A REVIEW ON THE IMMUNE SYSTEM -WITH EMPHASIS ON STRUCTURE, FUNCTION AND GENE ORGANIZATION OF THE IMMUNOGLOBULINS

By

RAMNATH SASISEKHARAN

BANGALORE

INDIA

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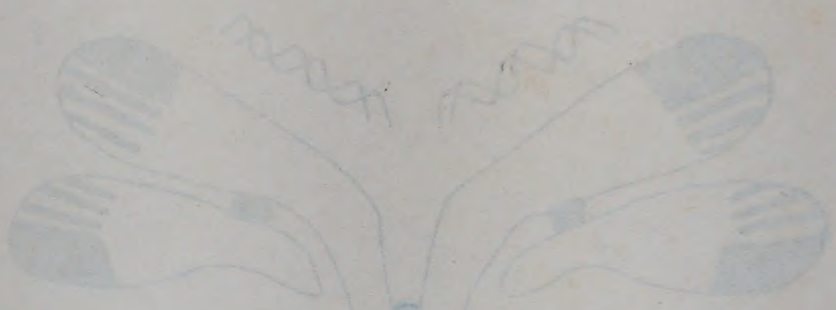

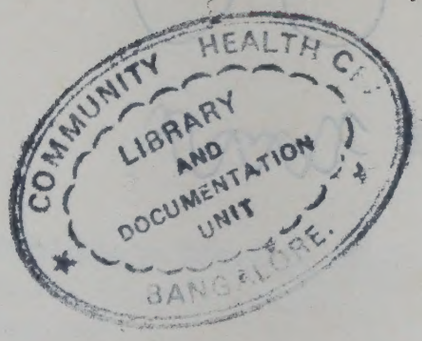
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RAMNATH SASISEKHARAN.

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INTRODUCTION

'Life is the maintanence of an equilibrium
that is perpetually threatened'

-Jules Bordet
1878 in the first ever book
on immunology.

Immunology by definition, is that branch of science which deals with the understanding of the 'ability' of a living organism to resist infection by any external agent.

The human body is endowed with impressive natural defenses against diseases. Consider the 'Black Death', the great plague of the fourteenth century. It attacked a Europe living in appalling filth, without any modern conception of cleanliness and hygiene, without plumbing, without any form of reasonable medical treatment - a crowded and helpless population. To be sure, people could flee from the infected villages, but the fugitive sick only spread the epidemics sooner and faster. Notwithstanding all this three fourth of the population successfully resisted the infection! Under those circumstances, the marvel is not that one out of four died; the marvel is that three out of four survived!

Thus originated the first ever concept of immunology: 'There exists natural resistance against any given disease'. This concept was known much before the discovery of the germ theory of infection. It was also known that recovery from illness was accompanied by the ability to resist reinfection. Thus the above concept was elaborated and stated as: 'There is also a thing as complete immunity against infection; it is usually inborn but may also be acquired. A single attack of measles or chickenpox will make a person 'immune' to that particular disease for the rest of his life.

The science of immunology which began, out of desparate need, thus developed and advanced due to the intrigued biologist's keenness in understanding the body's immune system. At first the interest had purely practical considerations. It was thought that the better, they understood how the body reacted in adverse conditions like that of an invasion by bacteria or attack by viruses, the easier it might be to make our immunological responses more effective against many other dangerous disease causing agents.

But, in the year 1954 English hematologists were excited when they discovered a women blood donor who

had two instead of a single blood group:about half of here red blood cells were A group cells and the rest O group. The physicians asked the women if she was a twin. Considerably suprised the women replied she was, but that her twin brother had died 25 years before at the age of three months. So here wasa women, who for a quarter of a century had been using red cells which 'really' belonged to her dead twin. In the process of trying to solve this mystery immunologists stumbled over a new concept; a different logic was applied in understanding the immune system. It was realised that they had grossly underestimated the immune system. Immunity really means much more than mere resistance against infection! By the modern definition immunology therefore is the branch of science which deals with the understanding of the ability of the body to maintain its integrity in such a manner that nothing disturbs the exquisitely balanced and self correcting mechanisms of maintenance and control essential to life.

It is believed that the immune system performs the function of maintaining the 'identity' of the body i.e. every organism has the capacity to recognize and identify cells or entities belonging to it, from those which do not. All those entities belonging to itself are termed as 'self' and those which are not as'non self! It is again

believed that the body under normal situations tolerates self entities, but mounts an immune response against nonself. The recognition of 'self against non-self' forms the central dogma of contemporary immunology.

In order to perform this function efficiently, the immune system produces chemical substances known as antibodies which perform two crucial functions: they are a)The recognition of non self; this function is performed by the surface antibody molecules known as, either surface immunoglobulins or receptor molecules. Once the surface antibodies recognize entities as non-self they elict, the appearance in blood antibody molecules known as secreted antibody molecules which perform the second essential function: b)the elimination of the non-self entities from the body. This phenomena is made possible due to the ability of the antibody molecules to specifically bind to the 'non-self' entities and nullify the disturbance they other wise would cause. This not only shows how important the antibody molecules are but also the responsibility they are endowed with in order to perform the functions of the immune system.

This review briefly analyses the immune system with special emphasis on the nature of the antibody molecules, and the progress that has been achieved in understanding

the structure, function and gene organization of the immunoglobulins.

Chapter I describes the history of immunology which certainly happens to be one of the most spectacular episodes in the history of science. Although immunology is as ancient as 1000 B.C., major .. milestones in understanding the immune system have been achieved in the last 100 years.

The second Chapter is a discussion of essential or classical immunology. This chapter deals with the general characteristics of the immune system. The discussion is divided into two heads, a)anatomy,which describes the structure of the elements of the immune system and b) physiology, which deals with the possible functions of these elements.

The third chapter deals with the basic structure, organization and function of the immunoglobulin molecule. Recent advances in the field, of crystallography and amino acid sequence analysis have greatly facilitated our understanding of the immunoglobulin molecules.

The fourth chapter dealing with the immunoglobulin genes discusses one of the most recent and major milestone in immunology. Recent spectacular developments in biochemistry, molecular biology and genetic engineering have been instrumental in our reaching the present state of understanding regarding the structure and organization of the immunoglobulin genes.

'The Spectacle of nature is always
new for she is always renewing the
spectators.....'

(Nature:Aphorisms by
Goethe Huxley 1869)

CHAPTER - 1

HISTORY OF IMMUNOLOGY

1.1. Introduction

The story begins as early as the 17th century, the time of smallpox disease, people in Turkey, India and China began to infect themselves with mild forms of smallpox , with the hope that they would become immune to its severe attack. This evoked various reactions some escaped and few died. It was a dangerous business because it was a measure of the horror of the disease that people were willing to risk the horror itself in order to escape from it.

The flock in Gloucestershire, England had their own idea about how to avoid smallpox . Superstitiously enough,these people thought that, those who had cowpox (a similar type of disease) would surely become immune not only towards cowpox but also smallpox !. Edward Jenner, a flock doctor cautiously studied this and thought that there was some truth in this flock 'superstition', and he inoculated an 8 year old boy with cowpox . Two months later came the crucial test. He then inoculated smallpox itself to assess the efficasy of the earlier exposure to the disease organism in preventing the disease. The boy did not contact the disease but developed immunity towards it. Thus evolved the first ever 'immunization' technique.

Jenner called the technique vaccination from 'Vaccinia', the Latin name for cowpox. Vaccination was soon widely acclaimed and spread through Europe like wildfire.

1.2. Immunotherapy

Attempts to discover similar inoculations for other severe diseases got nowhere for almost a century. It was Louis Pasteur who made the next big step in the year 1881. He discovered that a severe disease could be changed into a mild one by weakening the microbe that caused it. He acknowledged the depth of debt he owed to Jenner by calling his procedure vaccination eventhough it had no relation to vaccinia.

In 1890, a German doctor named Emil Von Behring, tried another idea. He wondered why one should take the risk of infecting with the microbe itself, eventhough it was in an attenuated form. Assuming that the disease agent caused the body to manufacture some defensive substance, he thought it would serve just as well to infect an animal with the disease causing agent, extract the defense substance that the animal produced, and inject the extracted substance into the human body. Indeed, Von Behring found that the scheme did work.

The defensive substance turned out to be in the blood serum and Von Behring called it 'anti-toxin' . He was able to produce antitoxins against tetanus and dephtheria in animals. The work of Von Behring forms the foundation to the modern techniques of 'immunotherapy'.

In 1894 Jules Bordet(who had written the first ever book on Immunology) and Pfeiffer, differenciated from the blood serum,a strange substance different from antitoxin. They termed it complement, which also seemed to participate in the defense mechanism.

In 1896 Durham and Von Gruber observed that the blood serum could clump bacteria. This observation thus formed the basis of identification or diagnosis of infectious specific clumping reaction and such a test was described in the same year by Widal for the diagnosis of typhoid.

The French Bacteriologist Gaston Ramon found that by treating the toxin of diptheria with heat or formaldehyde he was able to change the structure of it in such a way that it could be safely injected into a humam body. He called this as toxoid. Once toxoid was introduced in 1925, diptheria lost most of its terrors.

In the first quarter of this century the French biologists developed the priceless tissue culture techniques and that of culturing viruses. These techniques can be considered as one of the most important discovries in 1930's of science because the control of the ravaging yellow fever disease had its origins here! Bacteriologist found that by passing the disease causing virus through 200 mices and 100 chick embryo culture, a mutant virus could be obtained which would cause only mild yellow fever symptoms. Thus, total immunity against this disease could be achieved.

The next important technique that evolved was the use of antibiotics in tissue culture to grow a virus. In the 1940's John Franklin Enders, Thomas Weller and Chapman Robbins used this technique on the dangerous Poliomyelitis virus. In the late 1950's Albert Sabin developed a more effective method of using virus culture. He cultured various related polio virus strains but the ones that could not cause the disease as the original strain could, but those at the same time capable of producing antiviral substance in the body . The sabin vaccine gained a lot of popularity by 1960's and lifted the fear of polio.

So far, the discussion lay centred on the vaccination and the various techniques used with respect to vaccination - Vaccination being the founding stone of immunotherapy. There arose numerous other branches as mentioned earlier, of equal importance, which did help in the understanding of the biological significance of immunity and various biological events which are triggered on by foreign organisms or elements.

Thus it is seen, how important a place immunotherapy occupies in our understanding of the immune system. The major mile stones achieved by immunotherapy at various stages were vital to the extent of the survival of mankind! The other important credit that should be given to immunotherapy is that, various new fields in immunology and other biological sciences like chemotherepy, pharmacology etc have originated due to the development of immunotherapy in a very quick pace.

The major challange that immunotherapy and the other related branches of immunology are facing now, is the number one social problem as far as the diseases are concerned - cancer.

Cancer is actually a group of many diseases (about 200 or so) affecting various parts of the body in various fashions. But the primary function is always

the same: disorganization and uncontrolled growth of the affecting tissues. Its identification was done as early as the late B.C's during the times of Hippocrates and Galen. Uptil now what ever that has been achieved in understanding and preventing cancer has been of recent past (30 years or so).

Though our techniques have developed and advanced with respect to the immunotherapy, simultaneously new diseases have erupted. eg. the Legionaere's disease in 1978 caused much fear and uncertainity till a cure was found. The most alarming and fearful of diseases has been the AIDS(accquired immune deficiency syndrome), the origin of the disease is still not known and a cure is yet to be found.

Thus immunotherapy which has made so much of advancement right from its origin has still a long way to go.

1.3. Immunobiology

Coming back to the question 'What is that which makes vaccine work? Why are vaccines the way they are? etc. To answer these questions we go back to the early 1880's.

'If ever there was a romantic chapter in pathology it has surely been that of the story of Phagocytosis' - Lord Joseph Lister(1896).

This was so remarked referring to the Russian biologist Ilya Mechnikov. In 1883 Mechnikov discovered the white blood cells devouring Bacteria. He termed them Phagocytes, from his own observations and that of German pathologist Julis Cohnheim (who had demonstrated that WBC migrated from tissue capillaries to the site of injury). Mechnikov concluded that inflamation constituted an important defense reaction of the body and played a major part in bringing about recovery from bacterial infection. Thus arose the central idea of cellular immune response which was postulated by Mechnikov, explaining for the mechanism of immunity. Mechnikov's theory that Phagocytic cells destroyed bacteria met with immediate opposition amongst pathologist, who had often found phagocytic cells within the WBC of patients who had died of bacterial infections. They interpreted this to mean that these phagocytic cells merely provided transportation for the micro organisms, by carrying them to new areas of the animal body in order to spread the infection.

Pasteur's immunization against rabies, and the detection of bacterial substances in the blood serum by the German biologist Hans Buchner, had convinced the pathologists and others in related fields that 'chemical substances' what they called 'antibodies' are surely involved, and certainly phagocytes of Mechnikov's have nothing to do with immune reaction. The view that chemical properties of the blood have something to do against immunity were supported by the discovery of antitoxin by Von Behring. Following this was the theory proposed by Paul Enrlich - the 'side chain' theory explaining the antibody formation. All these formed the central ideas of Humoral response that was postulated explaining the immune mechanism.

The closing decades of the 19th century thus saw a spirited controversy developed between biologist who stuck to the idea that chemicals of the blood were involved in the immune reaction and those, a few followers of Ilya Mechnikovs who maintained that phagocytes mediated immune reaction. The gravity of the situation was intense as it turned out to be one sided right by the turn of this century, when the first ever Nobel prize in Medicine and Physiology was awarded to Von Behring for his work on antitoxins . An important

discovery came by the year 1903 when bacteriologists Wright and Douglas found that the serum of immunized patients contained substance that greatly activated the phagocytes engulfing bacteria. This substance turned out to be an antibody (named 'opsonin' later) . This discovery partly reconciled the two fighting groups to some extent by suggesting that both types of immune responses are important and that they act in concert. Nevertheless the controversy was unabated.

Humoral response concept had an upper hand right from the beginning. This one sidedness became more and more distinct with the discovery of few bacterial species which could not be engulfed by phagocytes alone, but required antibodies to do so. The phagocytes it was held, played only a secondary part; immunity depended mainly on antibodies.

1.3.1. Humoral immune response - A historical view

As mentioned earlier, in the year 1900, Paul Ehrlich proposed the side chain theory explaining the production of antibodies. He suggested that toxin molecules (antigen as they were known to be called as and still called) combined with pre-existing side chains on the surface of cells, thus stimulating them to generate

additional side chains which appeared in the serum as antibodies. Long before the chemists actually ran down an antibody, they were convinced that the antibodies must be proteins. They were sure of the fact that best known antigen were proteins and thus expected a protein-protein reaction . Only a protein (here antibody) could have all the subtility of structure necessary to combine specifically with an antigen.

During early 1915, the Austrian Pathologist Karl Landsteiner who had earlier discovered blood groups carried out series of experiments which gave positive indication to the idea that antibodies are very specific. The substance he used in order to elict an antibody response were not antigens, but much simpler compounds known as arsanilic acids in combination with a single protein. Landsteiner showed that a very small change in the structure of the arsanilic acid would be percived by the immune system which is reflected in the production of a characteristic antisera. Thus an antibody evoked by one specific type of arsanilic acid would not react with a slightly different type.

Landsteiner coined the term 'haptens' (from Greek - 'to bind') for compounds such as arsanilic acids which on becoming bound to proteins can elict an antibody but cannot do so on its own.

This non-biological antigen evoking an antibody formation appeared to create problems to the selective hypothesis of Ehrlich since it seemed difficult to contemplate that side chains pre-existed for a vast number of synthetic haptens. Thus importance to the side chain theory disappeared.

Between 1930-34 the understanding of quantitative precipitin reaction and that of proposal of Lattice theory of precipitation evolved; the biologist behind this were Heidelberger and Marrack.

In the year 1935, came out a new theory explaining for the production of antibody.- The instructive or template hypothesis. This theory was very popular for the next twenty years [see HAUROWITZ.F.(1973)] and suggested that a nonspecific antibody folded itself around a given antigen and possibly during protein synthesis gave raise to the stable specific antibody. Later experiments performed showed that this theory does not explain for antibody synthesis. [see Haber (1964), PNAS, 52, 1099]

The Swedish chemist Arne Wilhelm Karurin Tiselius in the summer of 1937 announced in the 'Transactions of the Faraday Society' of London, the development of

'electrophoresis technique' . This technique provides the most convenient and dependable means of analysing the protein content of the body fluids and tissues. Employing this technique to analyse the serum of blood, Tiselius found that the blood fraction known as globulin was in reality a mixture of three substances. He named them, the alpha, beta and gamma globulins. [The protein fraction separated according to difference in solubility and are classified as albumins or globulins].

In the year 1939, Kabat and Karurin Tiselius found out that antibodies were gamma globulins.

Purre Grabar and Curtis Williams Jr. combined electrophoresis and the antigen antibody reaction as a method of analysis of a single immunological protein. As a result of this a new technique evolved known as immunoelectrophoresis. This technique has a widespread usage in the study of immunology.

The immunotechniques thus did help a lot in the understanding of the antibody molecules. Coming back to the theories on the production of antibodies, suggestions that the body has some generalized protein molecule which can be moulded to fit any antigen were put forth - this idea had a good deal of support . (The antigen then acts as a template to shape the

specific antibody molecule) and was proposed in 1940 by Linus Pauling. It is seen that the various theories proposed in order to explain the production of antibodies were on the basis of Humoral response. Much of the head way (in understanding the antibody production) was made only in the late 1940's by Macfarlane Burnet and his group. [see section antibodies for details].

1.3.2. Immunobiology and allergy reactions

As far as the history of immunology is concerned yet another interesting branch of immunology has been 'unearthed' who's relevance and importance have been understood just under a decade or so.

In the early 1906 many of the bodies reactions like itching, sneezing, running nose etc. were discovered to be evoked by enteties like pollen, food fur, etc. This discovery was made by Charles Robert Richet. But not much was understood then so they were termed as 'anaphylactic shocks' and allergic reaction but in the last 20-25 years,much of its complexities have been revealed

In the 1940's, researchers found that allergy reactions are brought about by liberation of small quantities of a substance called 'histamine' in the blood. This led to the successful search for neutralizing 'anti-histamine'. 'Antihistamine' was seen to supress allergic reaction and did a lot to help in the therepy front. Thus it can be seen that theories on allergy and anaphylactic shocks became an independent branch of immunology involving specific immuno compounds.

1.3.3. Rediscovery of cellular response

Transplantation is a graft of tissue from one individual to another which was practiced since early times. This technique did not advance at all, because majority of the grafts were failures and seldom successes were a matter of sheer chance. But person to person transplant worked quite well in case of twins. The biologists thought that there must be some genetic connection for explaining this. [Transplants began with kidneys and went upto that of the heart. Transplants went on rage in 1969 but died down due to increasing failures]. Australian bacteriologist Mactarlane Burnet suggested that embroynic tissues might be 'immunized' to foreign tissues and that the free living animal might 'tolerate' the grafts. This did happen so, when Peter Medaware demonstrated.

The answer to how this works came in 1962 when Pirre Miller discovered, what one could say as possible reason for the ability to tolerate. He discovered that the thymus glands were responsible for tolerence , and also controlled the production of antibody producing cells. The role of thymus cells which were phagocytic was discovered. This discovery resulted in the rediscovery of 'cellular immune mechanism' and the importance of this mechanims was immediately appreciated. Thus immunology right from this point advanced with the help of both cellular and humoral response. Differenciation was made wherever necessary and co-ordination of both aspects has been instrumental for better understanding of immune system as a whole. Thus, to conclude, the history of immunology has been one of the most exciting episodes in the history of Science

'After you've heard two eye witness
accounts of a motor accident, you
begin to worry about history'.

-John McNab

Table - 1

HISTORY OF IMMUNOLOGY

A schematic representation of major achievements or milestones

Year	Milestones
1000 (±) AD	CHINESE AND INDIAN crude 'vaccination'
1798 AD	EDWARD JENNER Cowpox immunization
1878 AD	JULES BORDET First book on immunology
1882 (±) AD	KOCH.R Discovered delayed reaction-hypersensitivity
1881 (±) AD	PASTEUR LOUIS developed vaccination in the real sense application against hydrophobia.
1885 AD	ROUX AND YERSIN first described bacterial toxin
1886 (±) AD	ILYA MECHNIKOV discovered process 'Phagocytosis'
1890 AD	VON BEHRING discovered the use of antitoxins
1893 AD	BUCHNER discovered complement system
1894 AD	JULES BORDET discovered the involvement of complement and antibody in cell
1896 AD	DURHAM AND VON GRUBER described agglutination test for bacteria
1896 AD	WIDAL test for typhoid fever

THEORIES IN IMMUNOLOGY

ILYA MECHNIKOV
(Phagocytosis cell-mediated immunity)

CELLULAR

.
.
.

(Mechnikov rediscovered)

1944 MEDWAR AND BURNET
self and non self

1945 OWEN
chimerism

1947 LEVINE
Rhgroup system

1940 (±) instructive theory of antibody production

1952 agammaglobulinemia discovered

1955 JERNE AND BURNET
clonal selection theory

1961 (±) GOOD
discovery of role of thymus and Bone marrow

1970 (±) discovery of MHC systems

1906
VON PRQUET
'allergy' reaction

PAUL EHRLICH
(Antibody - Sidechain)
Protein molecule immunity

HUMORAL

LANDSTEINER
1900 discovery of blood groups

1901 WRIGHT AND DOUGLAS
discovery of opsonin

1930 HEIDELBERGER
immune precipitate reaction

1934 MARRACK
lattice theory of immune reaction

1939 KABAT
showed that antibodies are globulin proteins

1940 TISELIUS
Immunoelectrophoresis

1940 LINUS PAULING
proposed primary structure of proteins

1959 PORTER }
1960 EDELMAN }
primary structure of immunoglobulin

1965 DREYER AND CLAUDE
two gene one polypeptide theory of Ig produetion

CHAPTER - 2

ESSENTIAL IMMUNOLOGY

2.1. Immunology in the modern sense

A contemporary definition of immunity includes the ability of any organism to maintain its biological balance, if it gets disturbed due to the influence of the environment . . . . The organism-environment interaction results in various reactions, which includes disturbances of the biological balance with respect to the organism. These could be metabolic, physiological and genetic malfunctions etc. or invasion by external agents (chemicals or even other organisms). This definition is apart from the well known fact that immunity or an immune response is 'all those physiological mechanisms that endow the animal with the capacity to recognize materials as foreign to itself and to eliminate them without causing injury to its own tissues or cells.

So depending upon the type of disturbance that has been caused, the body of the organism mounts a reaction, the immune reaction to counterbalance the disturbance.

The immune reaction has been broadly divided into two basic catogories:

a) Specific immune reaction

b) non-specific immune reaction

In order to clearly understand the immune mechanism and that of specific response, the actual meaning of non-specific response has to be known first.

2.2. Immunobiology

2.2.A. Physiology

2.2. A-1. Non specific response

Non specific reaction can be considered as the general and formal reaction of the immune system, under any disturbed conditions, against the disturbance causing agent(s). This reaction is characterized by its 'typicalness'. In other words the immune reaction mounts initially the same reaction against any disturbance caused by the organism environmental interaction. Any disturbance in the 'equlibrium conditions' of the body could elict the non-specific response.

Two mechanisms constitute the nonspecific response. They are -

a) Nonspecific Phagocytosis

b) Inflammatory reaction

The nonspecific phagocytosis can be described as the most characteristic mechanism of the non-specific system (NSS). Phagocytosis is a process, carried out by special cell types, by which they engulf external bodies (be it molecules or even other cells). It is believed that the bodies phagocytic cells are capable of recognizing all those agents entering the

body (whether harmful or not). It is supposed that these phagocytic cells are made to 'see' and 'recognize' configurations within the body of the organism, wheather they are "self" or "non-self" i.e. configuration belonging to the body or those not belonging to the body. All non body configuration are seen as nonself. Immaterial of the nature of the configuration, if the phagocytic cells recognize these as non self, / they simply engulf the entity.

Thus, the mechanism of recognition of self against nonself also forms the basis of the initiation of the nonspecific phagocytosis (see figure 01). Once the nonself configuration initiates the non-specific phagocytosis, then this non-specific response triggers various other defense mechanism. Amongst the various mechanisms - 'inflammation' is one such event that is quite important. A spectrum of cellular and systemic events occur in which the host attempts to defend itself and maintain homeostasis. This reaction is generally referred to as inflammatory response. The classical signs of inflammation are well known, they include swelling, redness, heat and pain apart from the altered body functions. Generally (clinically) three stages of inflammation are recognized. They are acute, subacute and chronic inflammations. The

PHAGOCYTOSIS

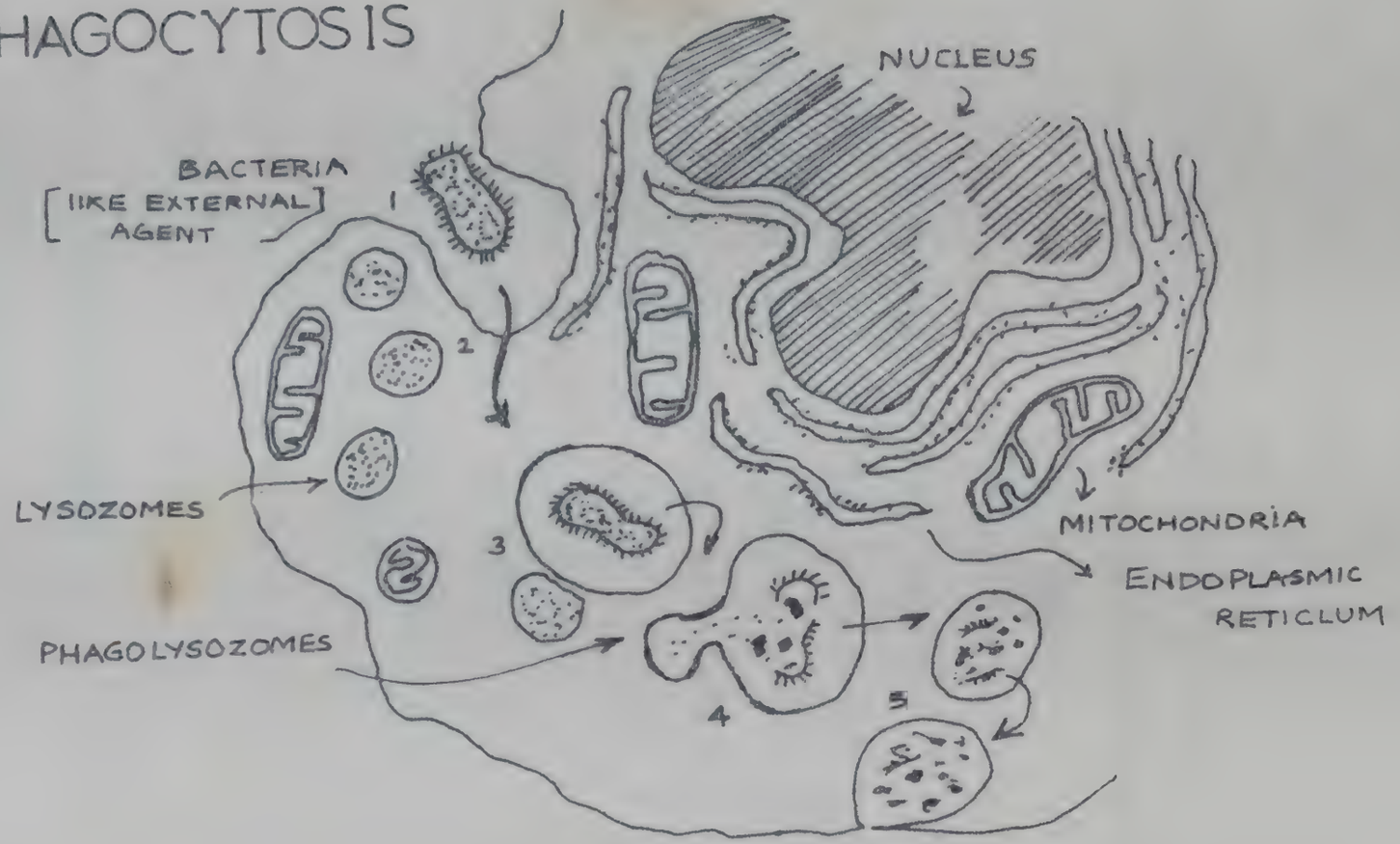


Figure - 01

acute inflammatory response is heralded by dilation of blood vessels and out pouring of leukocytes (white blood cells) This basically results in redness (erythema), swelling (edema) and frimness (induration) inflammatory response though protective has its limitations. Excess of accumilation of fluids results in damage of the associated organ etc. The subacute inflammatory response by definition can be said to be a delayed phase of acute inflammatory response and is characterized by the accumulation of the cells of the immune system and formation of what is called as granulation tissue. If the inflammatory response is not completely successful in restoring the equilibrium, the reaction may progress to a state called chronic inflammation. This reaction is characterized by the continued presence of all the immune cells like lymphocytes, monocytes and plasmacells. The chronic inflammation may result in functional impairment of the damaged tissues, Very little is known about how and what makes the inflammatory mechanism work (Florey H.W., 1970).

2.2. A-2 Specific immune system

It is usually felt, by experience and by that of the occurrence, that the body's defense system and stimulators try to bring normality and equilibrium after being

destabilized just with the help of the above two non specific mechanisms. But there exists situations - infact many, wherein the body is unable to tackle the destabilization in this phase. In order to tackle complex destabilizing or disturbance causing configurations, the body is endowed with a highly organized and more complicated network of defence parameters (most of our discussions will now concentrate on this phase of defense mechanisms). The reactions produced against agents by these parameters are very unique - they are only one of its kind to a given disturbance. It is also believed that each reaction and the agent causing their initiation have a one to one correspondence. All parameters involved in the above phase of defense reactions come under what is termed as the <u>specific immune system</u> (SIS). The most critical aspect of this specific response is its, basic nature and magnitude.

As mentioned earlier, the immune system has the capacity and capability to distinguish 'self' from 'nonself'. Entities which are capable of being recognized by the immune system as 'self or nonself' are collectively termed as <u>antigens</u>. There are two kinds of antigens as seen by the immune cells, the self antigens and the 'non self' antigens. The nonself antigens are capable of elicting immune response and thus termed

'immunogen'. The 'self antigens' are supposed to be as 'tolerated', no immune reaction against them are mounted normally.

The immunogens which could be a diverse array of entities (be it chemical molecules or tissues), elict the appearance, in the blood within a few days, of sizable amounts of proteins capable of specifically (chemically and structurally) combining with them. These proteins were termed as 'Antibodies'. This term was used much before their actual nature was known. Now a days all the antibody molecules are commonly referred to as Immunoglobulins. Antibody and Immunoglobulin (Ig) are more or less synonymous, although the term antibody still stresses its basis as an important biological molecule, 'Immunoglobulin' emphasizes the chemical nature of the same compound.

As mentioned earlier not all antigens are immunogens, this is due to the fact that some antigen though they react 'specifically' with antibodies, cannot induce the antibody formation . These molecules are termed as 'haptens'.

The specific response involves dual system that maintains defense against disturbances. The cellular immune response and the humoral response are the two

types. The cellular response is particularly effective against foreign tissues, intracellular virus. Parasites fungi etc and that of the humoral response against the extracellular bacterial and viral agents and even chemicals.

The cellular immunity involves cells of the lymph system, where as the humoral immunity involves antibody producing cells. This duality is due to the two populations of morphologically similar but functionally unique lymph cells called lymphocytes.

One such class of cells are known as T-cells (details described 2.2 C-1). They mediate the cellular immune response. The other class of lymphocytes are known as the B-cells which when activated mediate the humoral response by producing antibodies and memory cells.

In additon to these lymphocytes, the specific response involves many other kinds of active accessory cells [details described 2.2 B-1].

Their function include trapping of foreign substances in the body for presentation to the lymphocytes a and also includes mediation of physiological changes that accompany the immune response. The cells involved include phagocytes macrophages, granulocytes, platelets and modified lymphocytes amongst others.

General characteristics of the specific immunogonic response: Due to its uniqueness, the specific response is very complex and it involves multistage reaction bringing in various cells, chemicals and so on. But one can classify three general characteristics of the specific immune response that distinguish them from the non-specific reaction. They are a) specificity b) heterogeneity c) memory

Specificity: This aspect has been explained in the definition of specific response. The uniqueness of each specific response clearly characterizes specificity. Landsteiner first demonstrated this aspect using hapten molecules. Specificity could be further classified depending upon levels of organization eg. a) species specificity b) individual specificity c) organ specificity - intra organ and cellular specificity.

Heterogeneity: In case of heterogeneity, a vast array of cell types and cell products are introduced in order to interact with a diversity of disturbing agents. The variety of cells produced result in a multistep reaction involving various physical and chemical environmental conditions. The multiphase complex reaction involving various chemical substance including antibodies, marks the heterogenous feature of the reaction.

The heterogeneity results in the involvement of feed back mechanism - homeostatic control of the production of antibodies.

Memory: This is the most important of the characteristic features of the specific response. Memory is a mechanism by which a specific disturbance which had earlier elicted a response, elicts the same response again, but this time the response being much more effective, efficient and sharp. Memory process can be defined as the ability of the organism to register a disturbance along with the reaction that the body produces against it.

2.2. A-3 Function of specific and non specific systems

Analysis of the specific and non specific systems show that their functions could be broadly divided into three groups.

(i) Defense mechanism of the body
(ii) Homeostasis mechanism
(iii) Surveillance

Defense mechanism: This mechanism of the immune response mainly deals with the total elimination of invading external agents like micro-organisms. If the body's defense elements are properly deployed, then it will

sweep out the external agent. If the defense elements are produced in excess, then a hyperactive situation results. The organism may react against this hyper-activity and it would result in what has been called 'allergy'. If the defense elements are produced in deficit amounts 'hypoactive situation' results, which increases the chances of infections: disturbances of this sort are known as immunogenic disorders'.

Homeostasis: This is one of the most essential requirement of any multicellular set up and can be considered as 'typical' of multicellularity. It involves 'negative feed back mechanism'. Aberrations of homeostasis are examplified by 'autoimmune diseases'.

Surveillance: This is a recently discovered aspect of the immune system. This mechanism involves the screening of abnormal cell types which constantly move about the body of the organism. The immune cells and the para-meters connected to the system perform the function of recognition and disposal of these newly acquired configurations - usually through cell surfaces. Failure of this mechanism has recently been assigned a causal role in the development of malignant diseases - Cancer.

These are the basic function of the immune system (see figure 1.1) but there exists various immune mechanisms under these groups. And each of these mechanisms inturn are modified in accordance to all those factors which require different mechanisms under different conditions. There are a number of factors that modify the immune mechanisms: age, anatomic, environmental, genetic, metabolic, physiological, and microbial are those emportant ones (see Table 2).

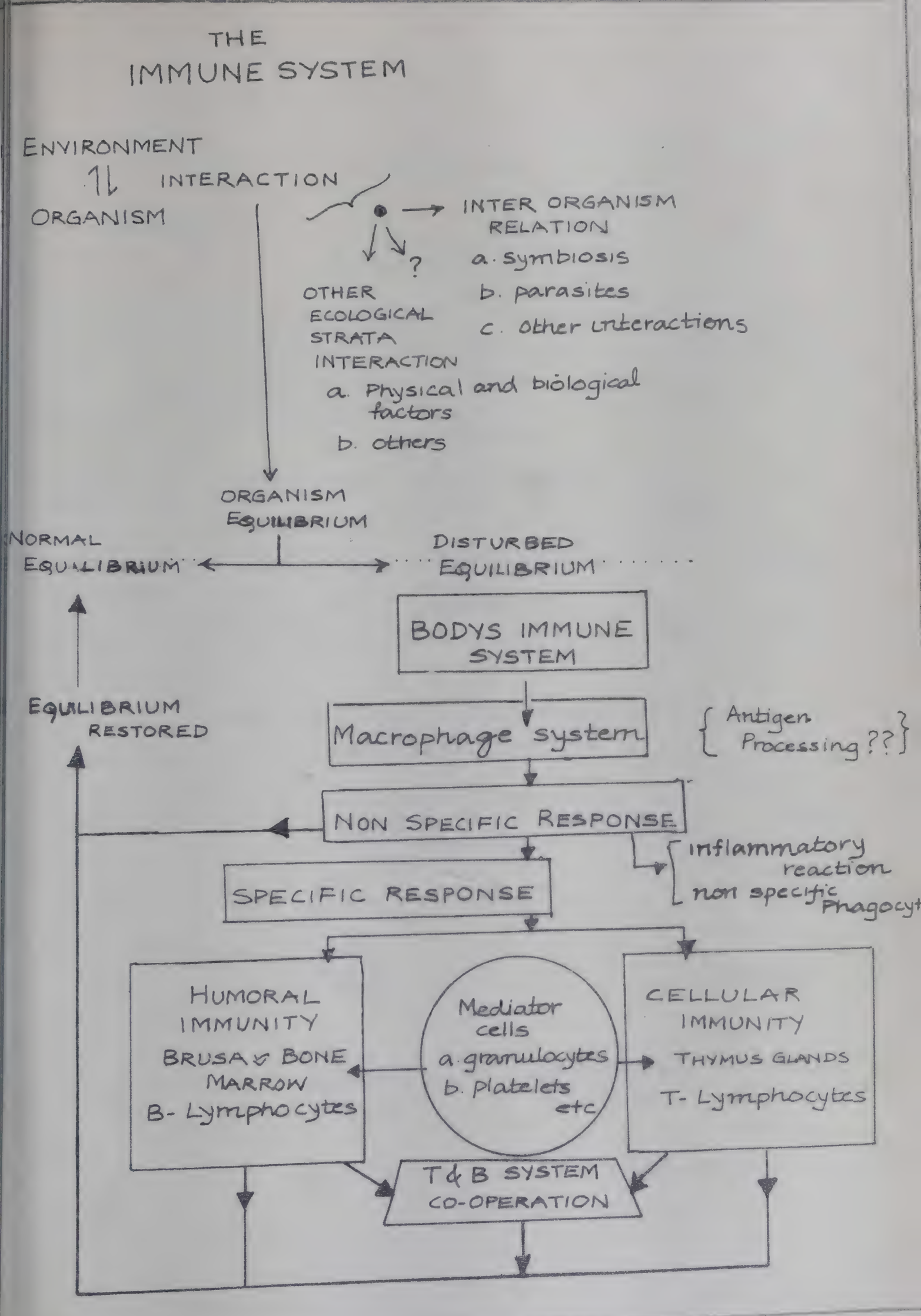

THE
IMMUNE SYSTEM
ENVIRONMENT
INTERACTION
ORGANISM
INTER ORGANISM RELATION
a. symbiosis
b. parasites
c. other interactions
?
OTHER ECOLOGICAL STRATA INTERACTION
a. Physical and biological factors
b. others
ORGANISM EQUILIBRIUM
NORMAL EQUILIBRIUM
DISTURBED EQUILIBRIUM
BODYS IMMUNE SYSTEM
EQUILIBRIUM RESTORED
Macrophage system
{ Antigen Processing ?? }
NON SPECIFIC RESPONSE
inflammatory reaction
non specific Phagocyt
SPECIFIC RESPONSE
HUMORAL IMMUNITY
BRUSA & BONE MARROW
B- Lymphocytes
Mediator cells
a. granulocytes
b. platelets
etc
CELLULAR IMMUNITY
THYMUS GLANDS
T- Lymphocytes
T & B SYSTEM CO-OPERATION

Figure - 01-1

Table - 2

FUNCTIONS

Immune response

Function	Stimulus	Example	Aberrations Hyper	Hypo
Defense	Exogenous	Micro-organisms	Allergy	-
Homeo-statis	Endogenous or Exogenous	Tissue injury death	Auto-immune disease	-
Watchdog function	Endogenous or Exogenous	mal-function cells mutation	-	Tumor Cancer

Elements involved in immunologic process for modification factors

Factors	Response	Encounter	Example
Age, Anatomic, Environmental, Genetic, Metabolic, Physiological	non Specific immune response	All	Phagocytosis Inflammatory fever, cellular immunity
Micobial	Specific response	Initial and subsequent	Humoral, cell mediated immunity

2.2.B Anatomy

In order to picture the immune system in a biological manner, the system may be viewed as a dynamic multi-compartmental collection of elements with ever changing morphologic components and functions.

Biologically immune stimulus are self-replicating, such as a disturbance by bacteria, virus or transplantation. Thus data obtained by laboratory experiments need not be the same under natural body conditons. The other essential point is the fact that not all configuration confronting the host are immunogenic i.e. lead to a specific response. Quite a few of these at first encounter the phagocytic cell (nonspecific response) and may be totally rearranged structurally (degraded), before becoming an immunogen elicting a immune response. (thus also initiating the production of specific Ig molecule against them). So in order to clearly picture the 'actual mechanism' of immunity, as far as possible, the anatomy and physiology of the system must be well understood.

A brief analysis of the anatomical and physiological aspect of the immune system are done subsequently(for complete description the excellent Volume by (Weiss.L. 1972) be referred).

2.2. B-1 Cell types involved in immune response

The vertebrates have a very complex net work of biological systems. There are many organ systems which in turn are made up of different organs. In order to carry out the functions of immunity, an independent system has evolved, the system being as complicated as the function they have to perform. The system is termed as the 'lymphoreticular system'. The organization of this system is very fascinating, as it somehow manages to reach every corner of the body and have its control over there. The cells of this system are well distributed right from tissue environment upto body fluids (lymphatic and vascular channels). The cells are housed within the blood, tissues, thymus,lymphnodes and spleen.[these regions are collectively termed as internal secretory system] and those body tracts exposed to the external environment: the respiratory, integimentry, gastrointestinal, genitourinary system/[these regions etc. are collectively termed external secrtory system]. (See figure 0.2).

The activation of the immune system,which is triggered by a stimulus,involves various cells and celltypes. The cell types involved in the nonspecific response are 1) mononuclear phagocytes a) granulocytes 3)platelets 4) lymphocytes.

The origin of these cells are from plurepotential hemato poetic stem cells located in the bone marrow and yolk sac of the fetus. The cell type differentiation and tissue localization of these cells are schematically present in fig. 03.

For simlicity, cells have been classified under three groups.

a) Phagocytic cell, b) Mediator cell, c) Lymphocytic cell.

Phagocytic cells: Phagocytic cells perform phagocytosis. They are also endocytic, because in addition to phagocytosis they perform pinocytosis (fluid droplets engulfment). In human, phagocytosis and pinocytosis are carried out by mononuclear phagocytes, neutrophils and to some extent by eosinophils

Mononuclear phagocytes (MNP) are group of cells which are widely distributed throughout the body and can effectively eliminate foreign material and derbis from blood, lymph and tissues. The (MNP) are produced from a stem cell in the bone marrow. They are produced as monocytes which transform into macrophage. The macrophage constitutes one of the most important cell types of the immune system as it plays a central role in various manifestations of the immune mechanisms.

LYMPHATIC SYSTEM

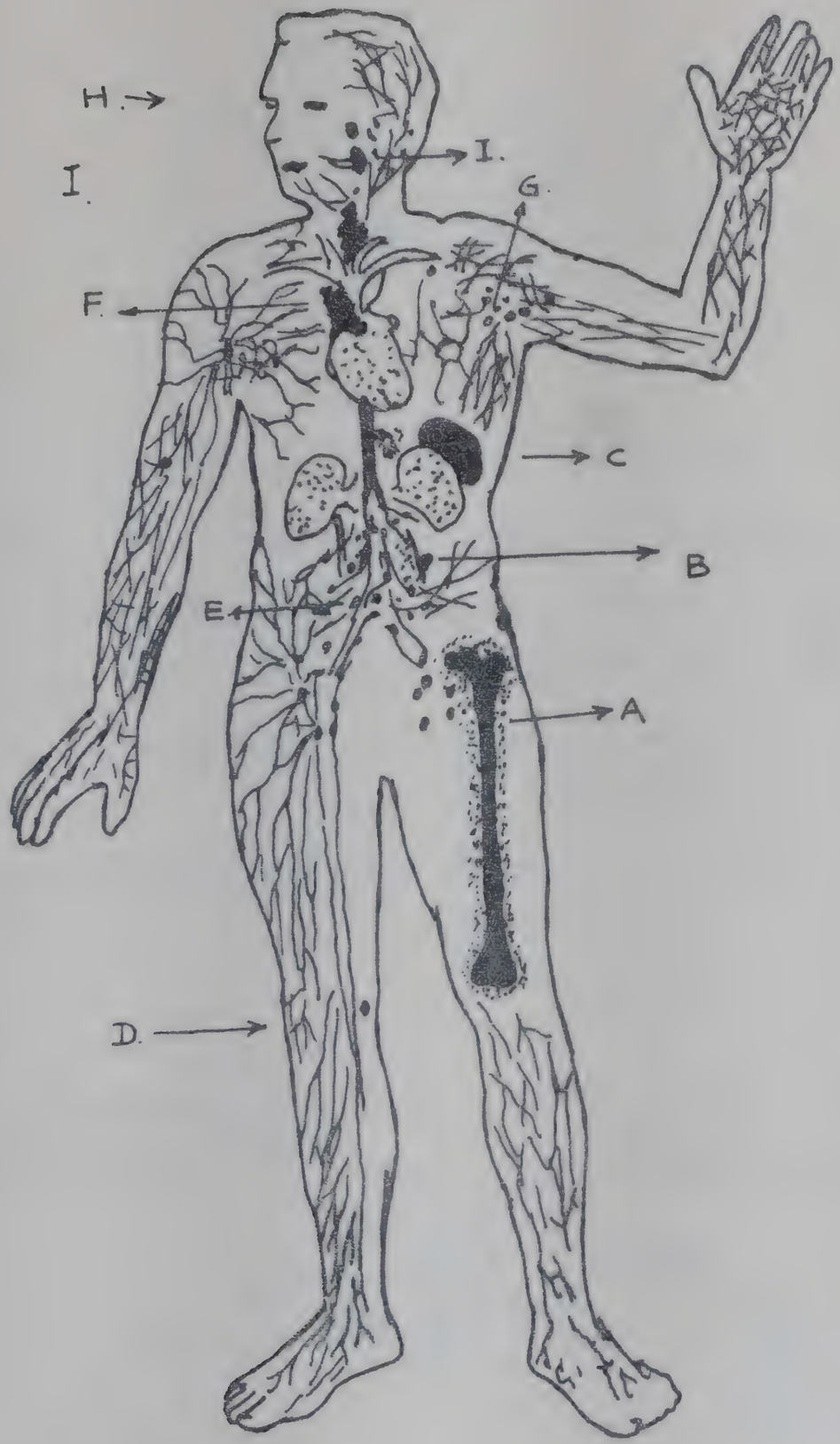
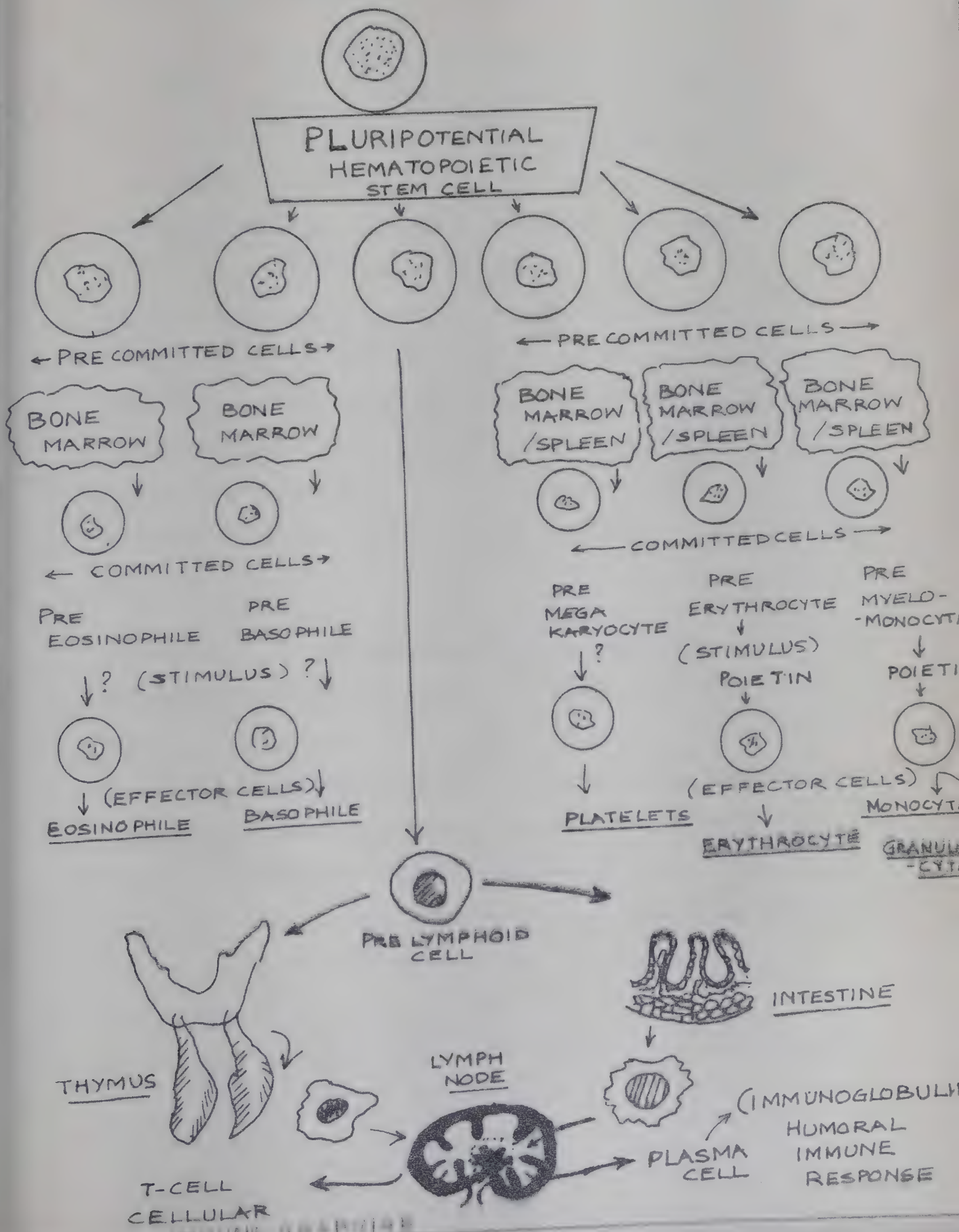


Figure - 02

* ONTOGENY OF IMMUNE SYSTEM *



The circulating monocytes are attracted towards the site of adverse reaction and this migration is known as chemotaxis. These migratory cells (monocytes) are activated by various chemical messanger. The activation results in transformation of these monocytes into macrophages. If these monocytes are sescile they are known as histocytes. Evidence indicates that various cell types derived from these are located at positions throughout the body and perform retention of antigen. Confusion exists concerning their classification due to their capacity to get modified or transformed from sessile condition into highly phagocytic cells. (Humphrey J.H. 1978 and Veerman A.J.P. 1974).

Neutrophils or Polymorphonuclear Leukocytes:

Polymorphonuclear Leukocytes as the name suggests are those cells having characteristic cellular structure and nuclear pattern. They possess a characteristic lobulated nucleus and cytoplasmic granules reactive towards peroxidase (details, Bainton D.F. et. al. 1976). There are three cell types known, basophils, eosinophils and neutrophils. Of these three, neutrophils and eisnophils have important role in immune mechanism. The origin of neutrophils is very distinct from that

of the macrophages.

bone marirow stem cells myeloid cell

myeloblast → promyelocyte → metamyelocyte →
bandcell → mature polymorphonuclear
leucocyte.

a) Neutrophil b)Basophil c)Eosinophil.

The maturation of these cells result in the appearence of two distinct classes of granules: the primary (azurophilic) and the secondary or specific granules. These granules contain myeloperoxidases and several other acid peroxidases. These granules, except for the secondary granules, are similar to lysozomes. Recent findings support the proposal that the activation of these granules is under the influence of the immune response.

Mediator cells

The second group of immune cells are the mediator cells. The basic function of these mediator cells are chemical in nature. They release chemical substances (known as mediators) that have a broad spectrum of biological activity e.g. increase and decrease of pH,permeatility of vascular vessels, influence on the concentration of ions etc. These mediator cells are groups of cells with

different structural basis but a common functional goal. This group includes mast cells, basophils, platelets, enterochromaffin cells and many others.

The Basophilic cell is a typical example of 'mediator cells'. The Basophile and platelets form the major population of mediator cells, chemicals like histamine, serotonin (a neuro transmittor) which have a broad biological activity are released due to the activation of these mediator cells. Basophil has a characteristic structure with well defined blue-black granule which are present in large numbers. The granules are claimed to contain mucopolysaccaride (e.g. heparin; MPS which are acidic polysacc arides). Their activity as chemical messengers for mediatory cell regulated functions is not well established as yet. This dual role of mediator cells is very essential for co-ordinating the functions of immune system. The co-ordination includes maintenance of a uniform pattern of reaction by the immune system; co-ordination of various mechanisms for a specific disturbance. Mediators are believed to play the 1) communication agent role within the immune system. 2) the mediator cells are believed to co-ordinate with the immune system, other biological system of the organism which are directly or indirectly involved in the immune reaction.

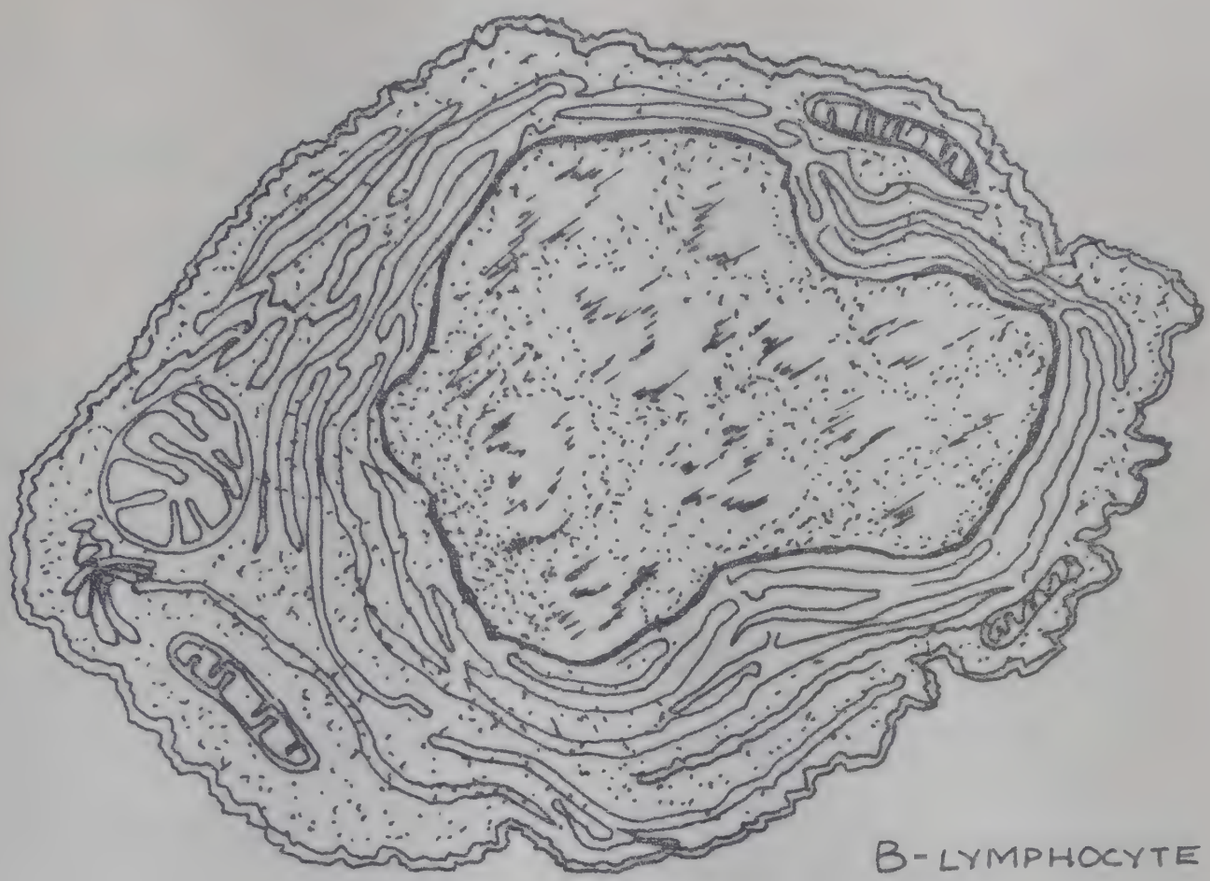
The third group and the most important group is the lymphatic cells; the lymphocytes.

The lymphocytes:

Lymphocytes are cells which are generally large having round nucleus with very little cytoplasmic material. They are known to perform the major role in the immune reaction (details of the physiological activities are described under a separate section). The present understanding regarding their formation is that their origin is said to be from the stem cells present in the yolk sac, the fetus and that of the bone marrow. These stem cells produce two precursor cells the rudimentary cells. These rudimentary cells give rise to the lymphocytes and erythroid, granular and poly nuclear cells of the circulatory system. Based on the migration of these rudimentary cells, lymphocytes fall under two catogories, they are T lymphocytes or T cells/the T-cells migrate from bone marrow through the thymus before populating the thymus dependent area of the lymphoid tissues/Ford C.E. et al (1966) and B-lymphocytes or B-cells which are precursors of the antibody forming 'plasma cells' mature in the bone marrow.

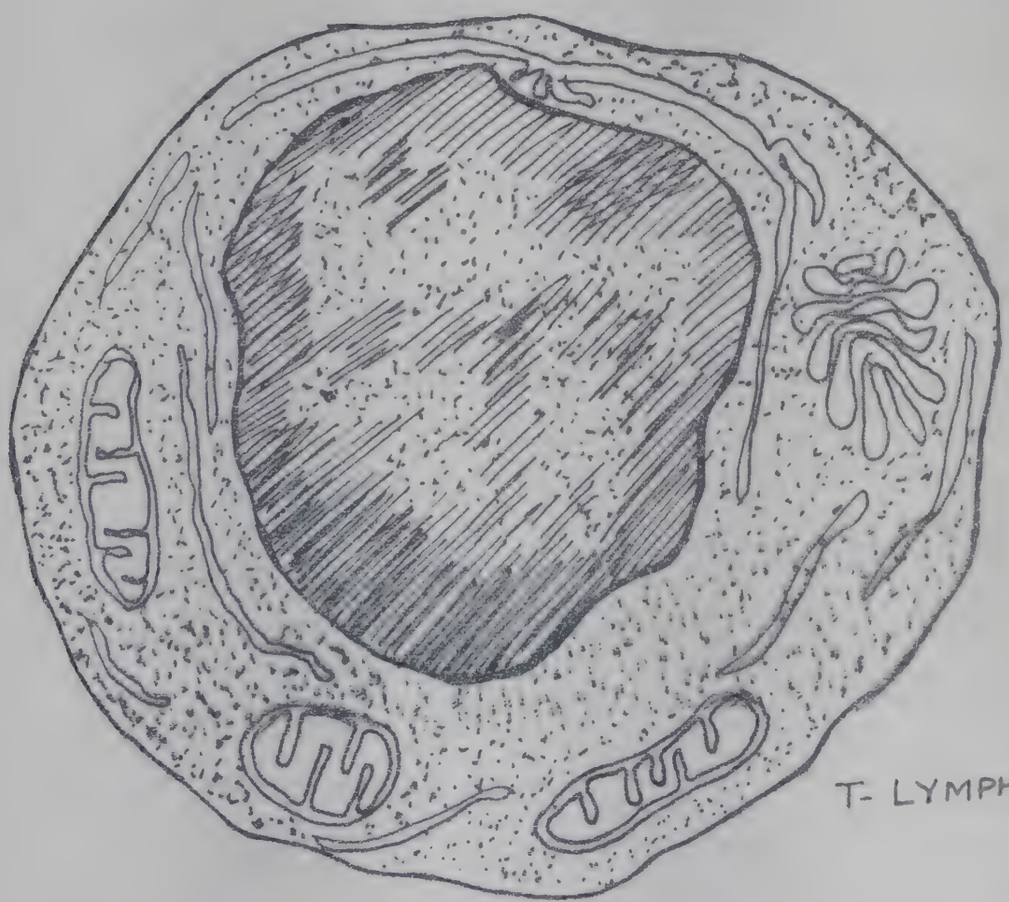
LYMPHOCYTES

A.



B- LYMPHOCYTE

B.



T- LYMPHOCYTE

Figure - 07 (A&B)

It was mentioned earlier that these cells are morphologically similar but differences do exist and these details have been described [Veerman, A.J.P. et.al. 1975]. The B-cells as compared to the T-cells are smaller and show many microvilli on their surface (hair like projections) and do not posses the smooth surface like that of the T-cells [Polliack, A. et.al,1974; Van Lwijk et. al.,1975 ;Bhalla, D.K. et.al. 1978.] Recent study involving scanning Electron microscope have revealed a great deal of the lymphocyte surface morphology for details [Kammerer, W.A. et.al. 1978] and [Bhalla, D.K. et al.,1978].(See figure 07)

When the lymphocytes are stimulated [antigenic or even nonantigenic] they transform themselves into blast cells and are known as lymphoblasts. These blast cells differenciate into small lymphocytic cell if derived from T-cells, or mature plasma cells and memory cells if derived from B-cells.

Before analysing the organ and organ systems connected with the lymphatic system, it is essential to note that endothelial cells form the lymphatic vessels connecting various lymphatic organs and also non-lymphatic organs. Detail analysis of the distribution of these cells have been done by [Pressman J.Jetal 1962; Marchesi V.T. et al. 1964; Anderson A. O. et al. 1976].

2.2. B.2. The organs and organ system involved in immune mechanism

Apart from the various cells mentioned, organ and organ groups also play a very essential role in immune mechanism. The organs perform, the role as sites of combat, some of them for storage of essential immune cells and others provide suitable environmental conditions for proper combat. The lymph cells are stored or located in areas of the body best suited to deal with disturbance and invasion

In order to perform the various immune mechanisms efficiently, the immune system organization is very critical. The organisation or net work of organs increases the probability of the foreign material encountering the respective immune agent of the organism. Therefore, certain organs such as the lymph nodes and spleen etc, contain an orderly system through which recirculation of blood and lymph occur. There are four such important immune organs which are integral components of the immune system. They are: a) lymph nodule b) lymph node c) spleen d) the thymus.

The lymph nodule are believed to be involved in exposing the antigen to the host, they are extensively distributed and well developed. When exposed to an antigen, they contain essential phagocytic and lymph cells. ..

The lymph node:

They are organs on the course of main lymphatic vessel, which basically perform functions relating to the feltering of lymph flow (Drinker C.K. et al:1934). The 'typical structure' of the node can be described as a beam shaped organ consisting of an outer cortex and an inner core known as medulla (Moe R,E. 1964) A fibrous tissue network known as reticulum exists [Clack. S.L. 1962; Fresen O. et al 1959; Moe R.E.1964]. The surface and the environment of these reticular cells support the macrophages and perform several of the functions. The lymph flows into the node from the afferent vessels. They proceed to the medulla and exit out via the afferent vessel. Blood is also circulated through the lymph nodes and its flow is in the opposite direction to that of the lymph. The circulation is believed to be closed, but evidences exist that fluid and cells are very frequently exchanged across the endothelial cells (Anderson, A.O. et al., 1975) (See figure 04).

The cortex of the node consists of closely packed lymphocytes, apart from the reticular tissue. Some of these clumps of lymphocytes are organized into 'follicles'. These follicles under 'immune stress' undergo

proliferation and are turmed germinal centres. Evidence show that the follicles are nothing but B-cells and outside the medulla in the cortex the same are made up of T-cells. The medulla contains a very high %, of plasma cells [Sorenson B.D. 1960].

The spleen:

The circulation of blood and lymph through the spleen has been known for a very long time. [Robinson W.,1926]. The spleen is differenciated into two regions called the lymphoid white pulp and erythroid red pulp. The white pulp function similar to that of the node (lymph) but the red pulp involves in a non-immune function of hematopoiesis. Detailed description of/ultra the structure of the vascular pathway is given in [Weiss,L.1972] and [Weiss,L.1974] and that of the organization of the spleen in [Galindo,B. 1963; Moore, R. etal.1963]. The main function of the splees appears to be blood cell destruction;thus its logical to suppose that they mount a major defense immune response against blood borne-antigens (see figure 05).

Lymphoid tissue distribution :

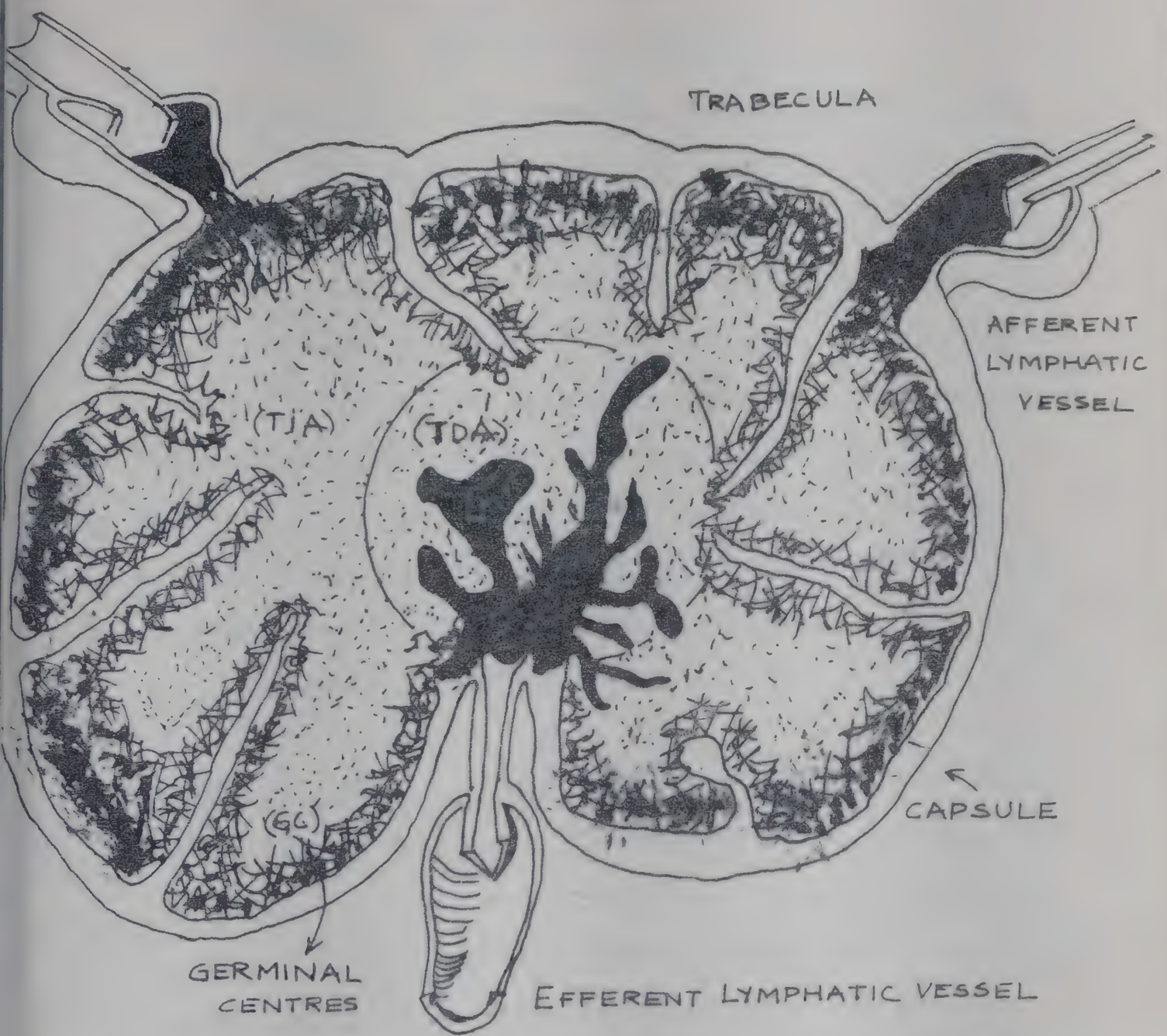
Throughout the epithelial tissues of gastro-intestinal tract, lung bronchial tissues, etc. the lymphocytes and plasma cells are found. In addition,

Figure : 04 : Schematic representation of a 'typical' lymphnode.

1. Thymic dependent area
2. Thymic independent area
3. Trabecula
4. Germinal centre
5. Subcapsular space
6. Capsule
7. Afferent vessels
8. Efferent vessels

The general structure of a lymph node. The cortical subcapsulor sinus (SCS) beneath the capsule of the node communicates extensively with the sinuses of the medulla. The subcapsular sinus drains the extracellular space via afferent lymplatics and is lined with phagocytic cells. The primary follicle (PF) lying directly under SCS in the cortex is an ovoid accu mulation of small lymphocytes lying in a mesh work of dendritic reticular cells. The secondary follide(SF) is composed of the mantle(M) the components of which are similar to those of primary follide and the germinal centre(GC), which contain small and large lymphocytes, blast cells and macro phages and reticular cells. The diffuse cortex(DC) includes lymphocytes, macrophages and post capillary venules(PVC) . The medullary sinus(MS) is lined by phagocytic cells. The medullary chords(MC) are close-packed inter connected spaces containing plasma cells and lymphoblast. [Adapted form Immunology by Hood et al Benjamin and Cummngs Calif, USA].

I. LYMPH NODE



THYMIC DEPENDENT AREA → T-CELLS

THYMIC INDEPENDENT AREA → B-CELLS

II. SCHEMATIC REPRESENTATION OF LYMPH NODE

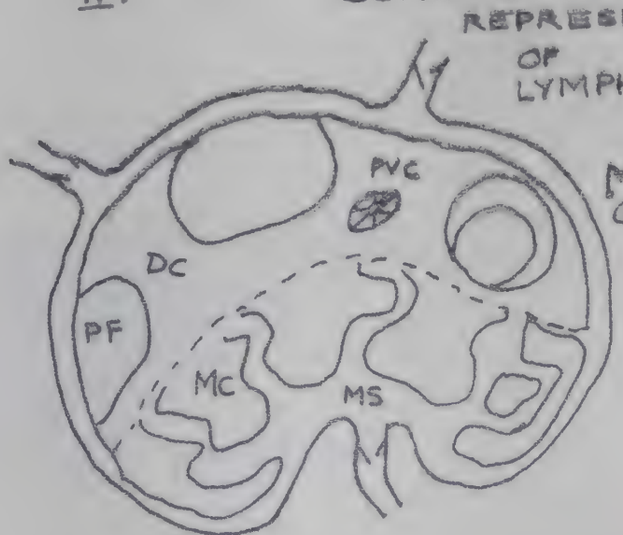


Figure - 04

SPLEEN :-

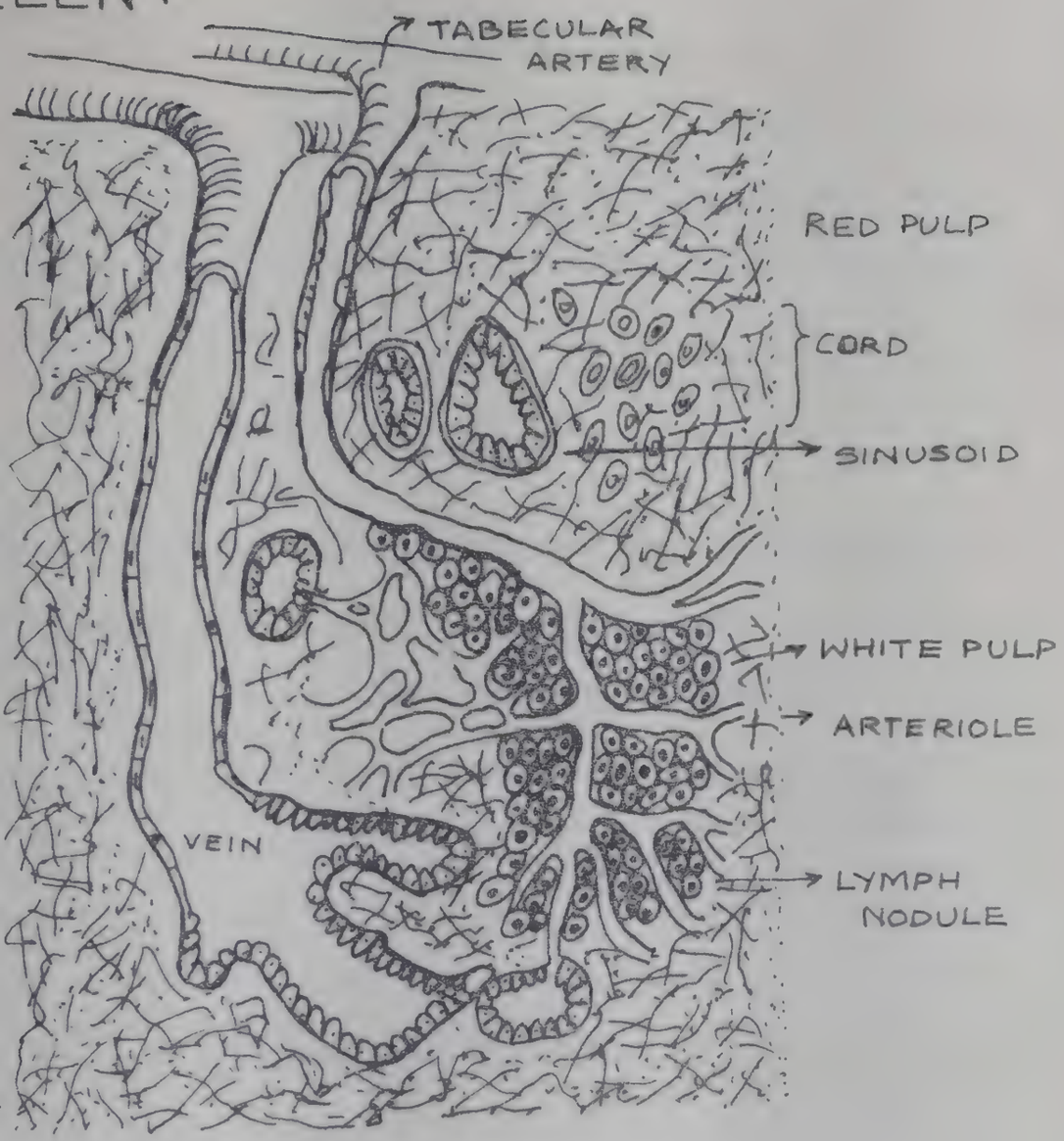


Figure-05

THYMUS :-

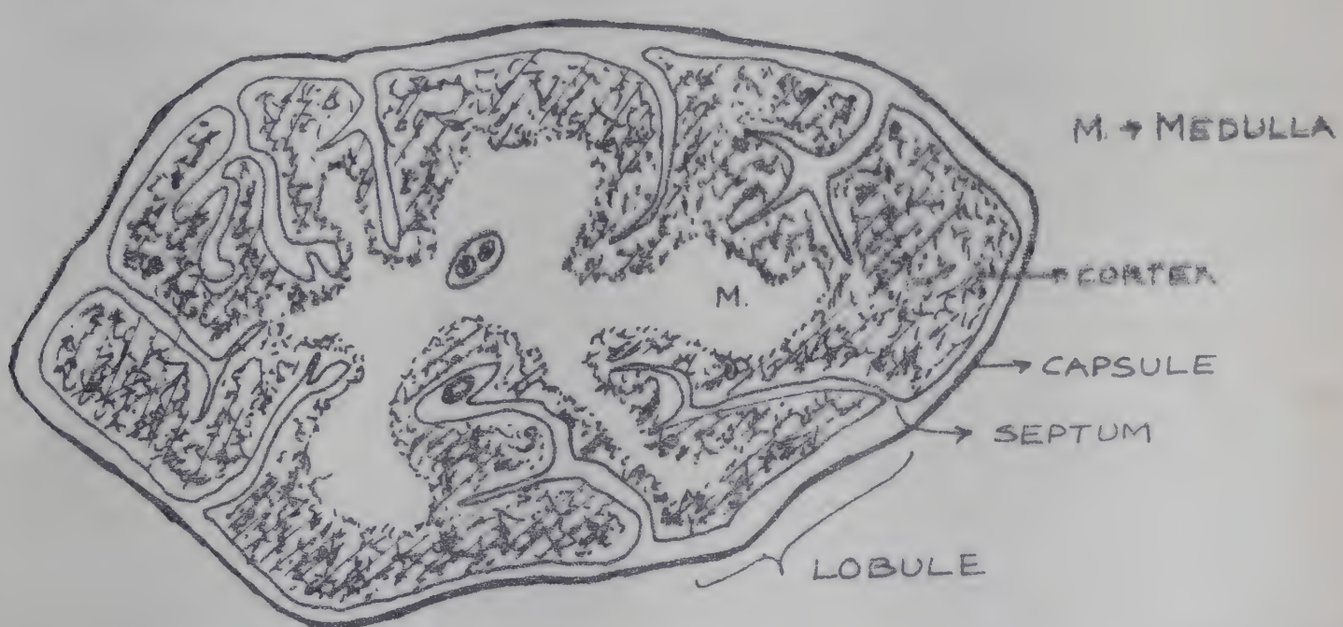


Figure - 06

structure such as peyer's patches, the appendix, the tonsils etc. contain lymphoid follicles and germinal centres similar to that of the node and spleen (see for details [Guy Grand, D. et al 1974; Jeol, D.D. et al 1971].

Thymus:

This organ remained a mystery as its actual function was was not known until recently. The gland was found to be a very essential and important organ as it played the part of being a master organ in the young and in tuning on the complete lymph system of the organism for rest of its life. They are stimulated to produce lymphocytes which take part in the cellular immune mechanism. Two major functions have been attributed to the thymus (1) that it acts by the elaboration of a hormone which expands peripheral component lymphocyte population (2) that it acts by direct seeding of peripheral lymphod tissue with lymphocytes (see figure 06). [Miller, J.F.A.P., (1961); Weissman, I.L.(1967)].

2.2.C. Immunophysiology

The answer to a possible question of what happens to any foreign body injected into the organism is one of the most interesting answers of immunology. It is seen that the immunogen (the configuration has since elicted an immune response) either remains at the site of administration or is localized in the draining lymph node. There is no specific pattern of distribution, when immunogen is intra-venously injected as they appear to be spread randomly in the circulatory system. The next step is the taking up of these immunogens by the phagocytic cells (mononuclear phagocytic system).

The macrophages in the lymph nodes and spleen engulf the immunogen, if the organism has not faced this immunogen earlier. If the organism had faced the immunogen previously, then the injected immunogen can be found associated with dendritic macrophages of the lymp node. This result has brought in a lot of questioning in our understanding of 'these stages of immune mechanism'. Many group of immunologists believe that the phagocytic work of macrophages limit with the engulfment of the foreign particle, but many others propose that macrophage 'processes' the immunogen before

elicting a response. The above result of the location of immunogen support the latter concept, but still a lot of controversy remains.

2.2.C-1. The lymphocytes

As mentioned earlier, the lymphocytes are the major cells of the immune system. They are produced by the primordial lymphocyte precursors which arise in the blood island of the yolk sac and then migrate to the embryonic liver and bone marrow.

They permanently settle down at the bone marrow producing the blood forming stem cells which have lymphocyte precursors among there progeny. Based on the migration of these cells, the lymphocytes thus produced fall under two categories. The T-cells or T-lymphocyte and the B-cells or B-lymphocyte (see figure 07).

Maturation of both T and B lymphocytes are believed to include an antigen independent phase and an antigen dependent phase. The differenciation steps that occur in the fetus or in those immune organs that are not processing foreign antigens are thought to be antigen independent . The lymphocytes differenciating in these environments become 'immunocompetent' and are known

as virgin lymphocytes. These cells undergo antigen stimulated differenciation and result in the generation of memory and effector cells.

The maturation and production of T-lymphocytes involves at least four distinct events.

a) Establishment of an appropriate environment in the thymus

b) Seeding of the environment by the stem cells

c) Intra thymic cells proliferation and differenciation into virgin cells

d) Migration of these virgin cells to various parts of the immune system.

Maturation of B cells also involves antigen independent induction by a micro environment. Similar to T cells, the B cells acquire specific homing and cell recognition properies as well as antigen specific cell surface receptors which are presumably Ig of a specific type. Very little is known about the early maturation of B-cells, except that they include membrane bound, Ig molecules of a specific kind. Following their maturation in the bone marrow, the antigen specific virgin B cells migrate to periphical B cell domains where they respond to further

stimulation and differenciate into memory B cells and antibody producing plasma cells.

Much more has to be understood regarding the T and B cell differenciation. Many of the cancers appear to be of either T or B cell origin, but they exibit different surface bound molecules. Example of acute lymphocytic leukemias being T cell dependent where as chronic form of the same cancer is due to B cell proliferation.

2.2.c.2. Antibody

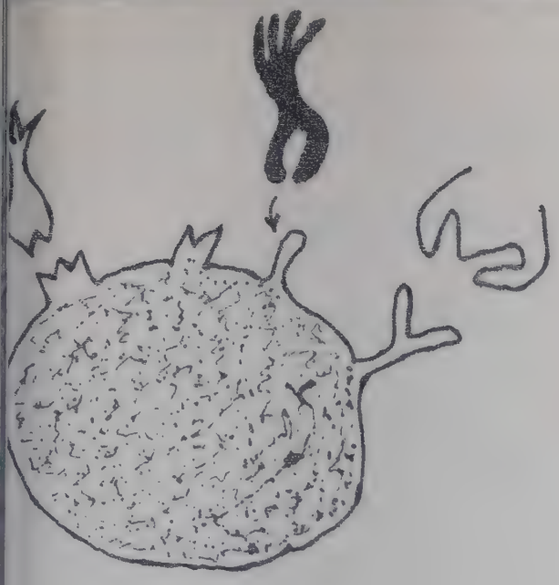
It is generally accepted that the antigen binding to lymphocyte receptors triggers an immune response. The antigens which are engulfed are incorporated into special vacoules, called 'Phagosomes' of the macrophages. Binding of any antigen to the surface receptors of either T or B lymphocytes trigger the blast transformation and other reactions mentioned earlier. The importance of the blast transformation is that of the morphological re-organization of the lymph node. The B cells produce the plasma cells (see figure 8B) which then synthesize 3000 to 25000 antibody molecules. Again the important aspect of these 25000 molecules

are that, both the specificity and heterogenity of the molecules are maintained.

One of the central question in immunology which arises here is that how are the antibody molecules produced? what are the factors which trigger their production. As mentioned earlier, the ability of the body to mount specific response has fascinated immunobiologist right from the turm of this century.

In 1900, Paul Ehrlich proposed the first selective theory of antibody synthesis. He proposed that the antigen molecules combined with the pre-existing antibody (then termed side chain) expressed on the surface of the cells, thus stimulating them to produce more of side chain molecules (see figure 09). This theory fell into disrepute when Landsteiner (1944) shcwed that the proposed hypothesis was highly improbable biologically.

From 1935-1955 the instructive theories gained popularity and were generally accepted (details; Haurowitz, 1973). These theories proposed that the antigen molecules acted as template for instruction, which the immune cells would follow and thus produce



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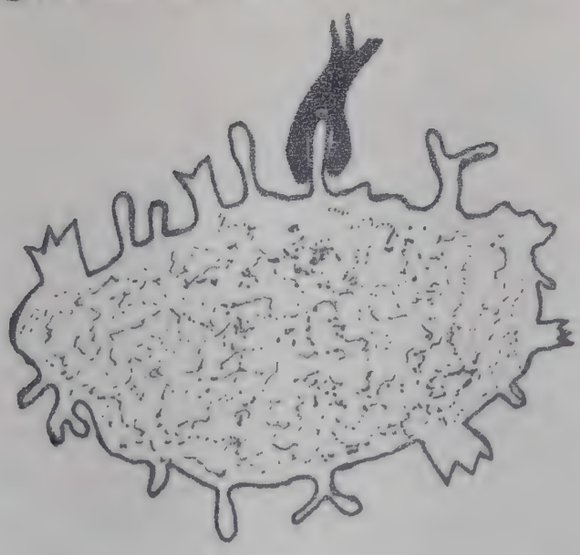

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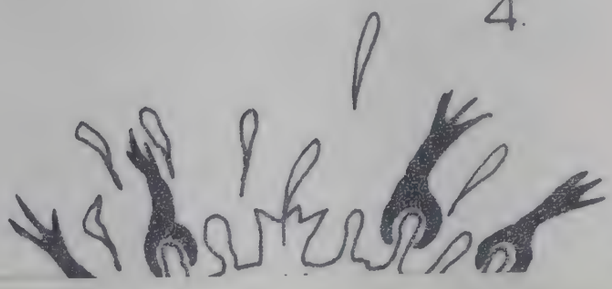
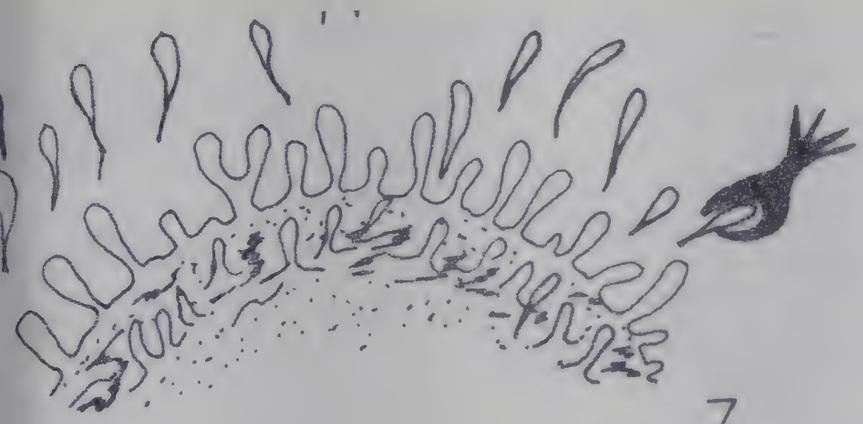


Figure : 09 : Schematic representation of Paul Ehrlichs' side chain theory of antibody formation (proposed in the 1900's). The antigen (black) combined with its corresponding side chain or receptor finally resulting in the excess regeneration of receptor (reproduced from Croonian Lecture, 'On Immunity with special reference to cell life', Proc. Roy. Soc. London (Biol)66; 424,1906.



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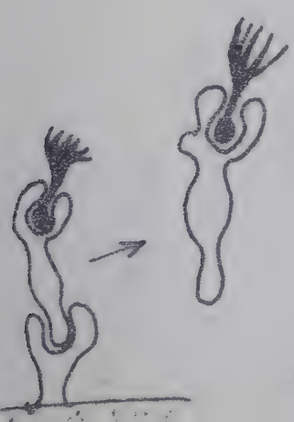


8.

Figure-09

THEORY-ANTIBODY PRODUCTION

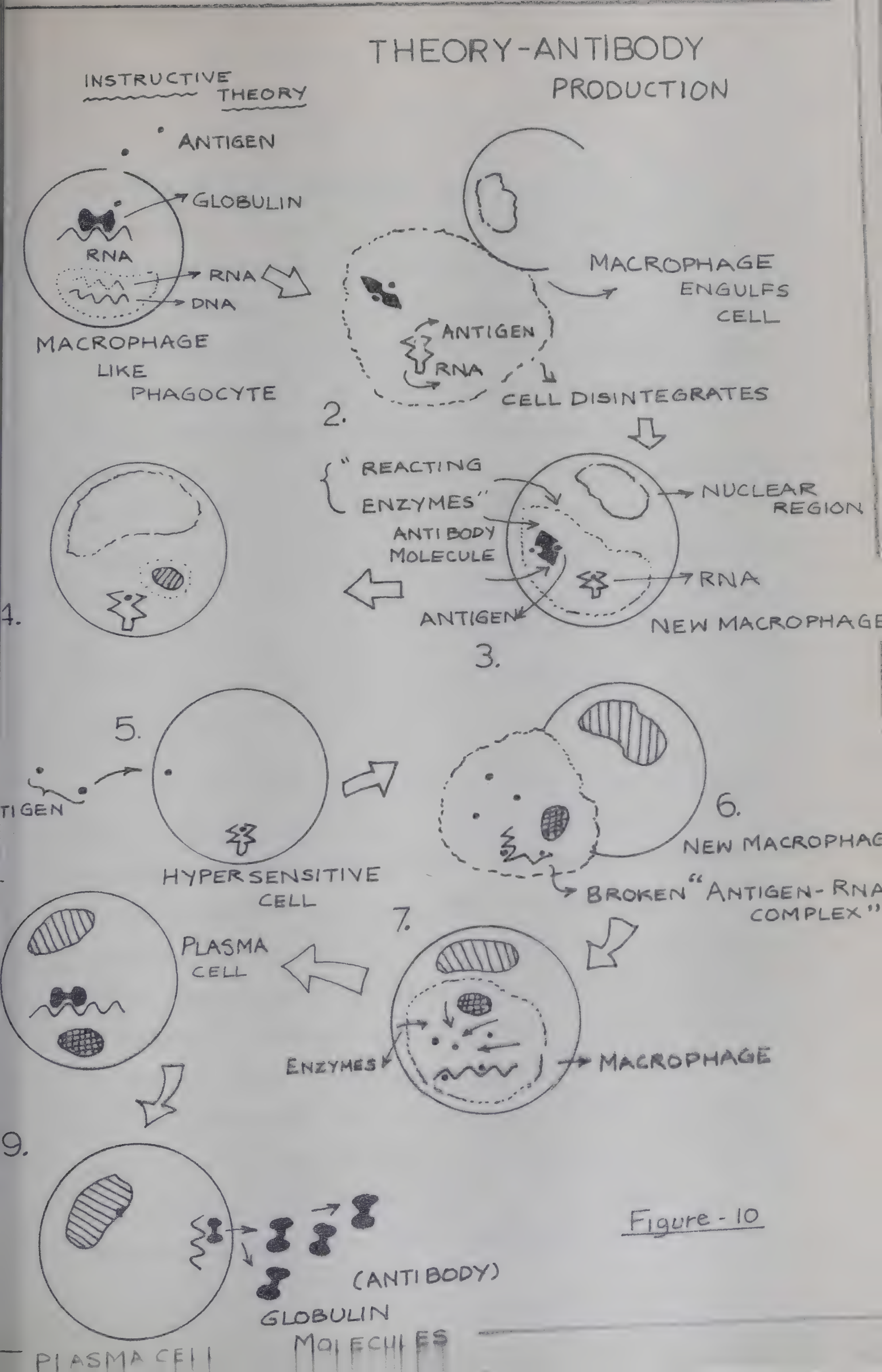


Figure - 10

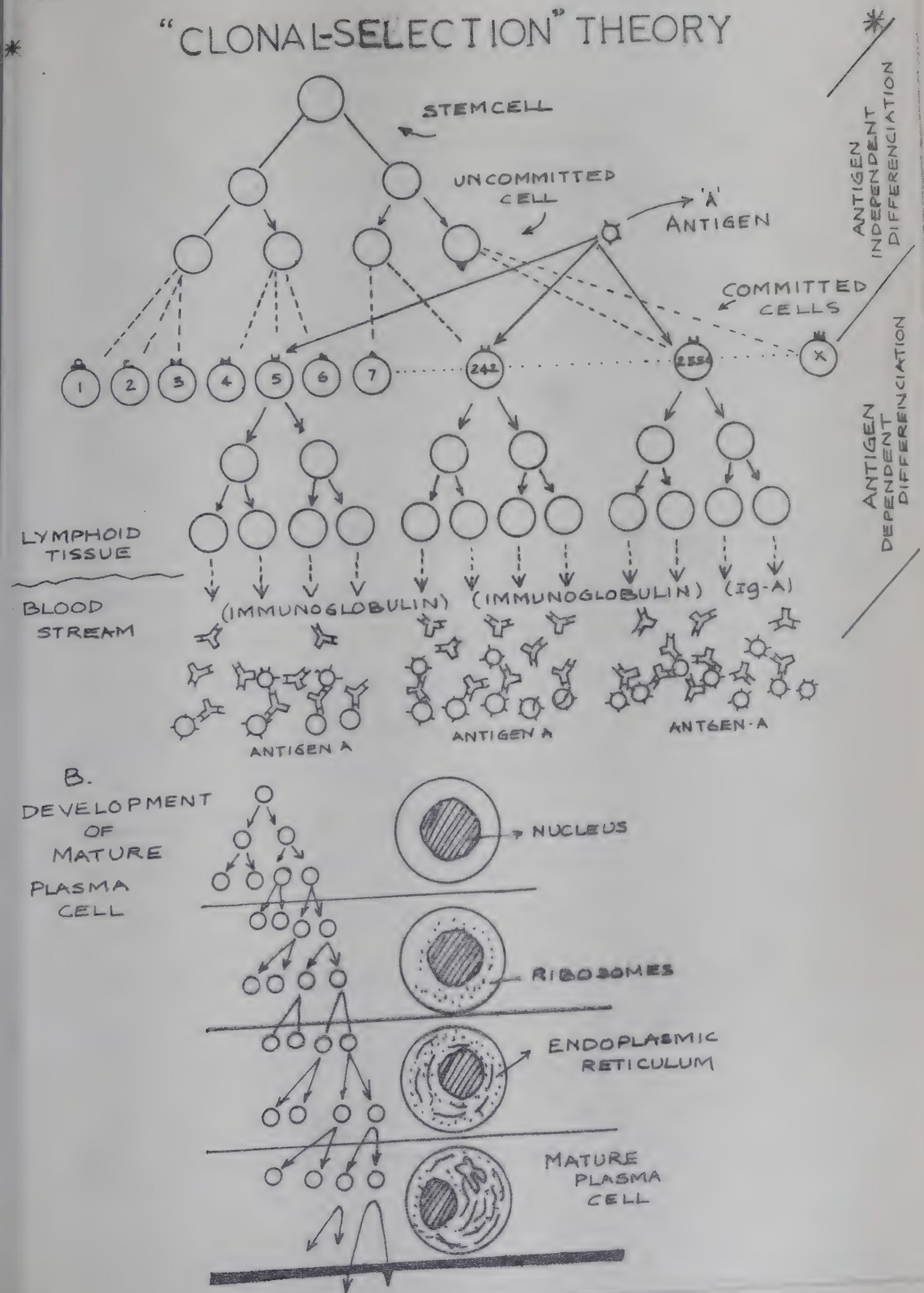
specific antibody molecules (see figure 10). The contral idea of the various hypeses consisted of the situation wherein a nonspecific protein molecule (gamma globulin) folded itself around the antigen and thus resulted in a stable structure of specific antibodies. The basic idea came under sharp questioning when it was discovered that the structure of the nonspecific gamma globulin depended upon the amino acid sequence and not any random molecule (Haber,1964) . Indirect template hypothesis was proposed arguing that the antigen specificity may be converted into a DNA sequence which then codes for the corresponding antibody. The details of instructive theory are given by (Haurowitz,F.,1973).

In 1955, Jerne proposed the first modern selective theory which thus turned out to be renaissance of the original selective hypothesis. The central concept of the selective theory was that the diversity of receptors involved in the recognition of the antigen arise spontaneously in the absence of antigen. Burnet (1957) and Lederberg(1959) proposed a modified version of the selective theory which argues that these pre-existing receptors were associated with cells which had undergone specific clonal differenciation on meeting the antigen. This became known as the '<u>clonal selection hypothesis</u>'. This hypothesis now is largely

accepted. The basis of this hypothesis is that, the lymphocytes are said to express surface receptors for a specific antigen and this is so before the antigen enters the immune system. Each lymphocyte is said to carry a unique receptor (antigenic receptor). These cells originate (as mentioned earlier) from a stem progeny which undergoes an 1)antigen-independent differenciation and result in 10^5 to 10^8 clones of cells which in turn are capable of producing specific antibody molecules. A typical lymphocyte expresses on its surface the 'specific' antibody molecule that it is capable of producing 2) the second stage of antibody production is the antigen dependent stage, which leads to differenciation of specific clones (the one which reacted with the antigen) and thus a large amount of the specific lymphocytes, which are capable of producing the required antibody, are produced. (see figure 08A). Three lines of experimental results have added support to the clonal selection hypothesis. The interpretation of the results are as follows:

a) animals injected with highly radio-active antigen produced lymphocytes capable of specifically binding to these antigen molecules, but due to high radio-activity they were irradicated and killed. Another set of antigens (different from the first) injected did succumb to the immune response. Thus the killed cells only corresponded to the specific antigen(radioactive).

A.


"CLONAL-SELECTION" THEORY
STEMCELL
UNCOMMITTED CELL
'A' ANTIGEN
ANTIGEN INDEPENDENT DIFFERENCIATION
COMMITTED CELLS
ANTIGEN DEPENDENT DIFFERENCIATION
1
2
3
4
5
6
7
242
2554
X
LYMPHOID TISSUE
BLOOD STREAM
(IMMUNOGLOBULIN)
(IMMUNOGLOBULIN)
(Ig-A)
ANTIGEN A
ANTIGEN A
ANTGEN-A

B.

DEVELOPMENT OF MATURE PLASMA CELL
NUCLEUS
RIBOSOMES
ENDOPLASMIC RETICULUM
MATURE PLASMA CELL

b) through a column containing certain specific antigen fixed chemically many lymphocytes were passed through. The cells capable of interacting to that antigen bound to it and the other lymphocytes came down the column . This showed that there existed specific antibody producing cells.

c) The third is one of the most important result as it not only explained this hypothesis, but also gave an insight to our understanding of the 'immune-clumping'.

It was found that immune cells (B-cells) possessed surface Ig. If an Ig specific antigen is added and if this antigen is multivalent, then it makes the B-cells to cap. 'Capping' is a process in which all Ig molecules of the B cell are drawn to one end of the cell and it forms an immune lattice. The immune clump, is later engulfed by the macrophages. This result shows the existance of surface antibody molecules which the B cells bear as samples of the one's they produce.

The clonal hypothesis is much accepted due to the fact that it has also postulated an explanation regarding one of the most puzzling aspects of immunology— the concept of 'tolerance'. Tolerance can be defined as the failure of an organism to mount an immune response

against a given specific antigen. It is said that the organism is tolerant of its own antigens - the capacity to recognize self and foreign. This mechanism is still unclear but a consequence of this mechanism is that, those lymphocytes which are specific to the self antigen are made inactive with the help of some mechanism. The mechanism is believed to occur some time during early stages of lymphocyte differenciation in the bone marrow and thymus and is referred to as 'clonal abortion'.

Thus tolerance remains to be one of the unanswered questions of immunology and that the clonal theory being the only theory which has simultaneously explained many immunologic/al events hypothetically with out much flaw.

There are five classes of antibody molecules. They are a) gamma immunoglobulin IgG b)Mu immunoglobulin : c)Epsilon immunoglobulin:IgE d)Alpha immunoglobulin: IgA and e)Delta immunoglobulin:IgD. Each of the different Ig molecules are found in the serum with varying concentration and 'activity span'. Below are described their salient biological features. Their structural details are described in the next chapter. The Immunoglobulin Mu (IgM), the first antibody produced against any immunogen, is a complicated molecule structurally. They are highly effective molecules and show

high efficiency in capturing immunogen. The IgM are found generally in the blood(Due to the largeness of their structure they move very slowly). They are largely expressed on the surface of lymphocyte cells.

The Immunoglobulin Gamma (IgG): these monomeric antibody molecules are produced later, in the immune response, after the IgM. They are the most prevalent antibody in the blood and largely found in tissue spaces. They play a very important role in the 'complement mechanism' of the humoral response. These Ig molecules activate many other parameters which are involved in the immune mechanism. They are the sole molecules capable of providing immunity to the fetal organism.

The immunoglobulin alpha (IgA): are also produced much later in the immune response, and occur either as a monomer or as a dimer. Some times, they exhibit the trimer configuration. The IgA are thought to act as barriers against microorganisms at junctions of the body easily exposed to the environment. (nose, ear, mouth etc.) IgA is a major molecule present in the saliva, sweat and tears.

The Immunoglobulin Delta (IgD): are not widely distributed but they are extensively expressed on the surfaces of lymphocytes. They rarely occur in

free state, therefore not much is known about their functions.

The Immunoglobulin Epsilon (IgE) are the most strangest of the Ig molecules because of their characteristic nature of mounting reaction against 'strange disturbances', even though they are found in the serum in almost negligible concentration, they stimulate the mast cells to degranulate and result in the production of heparin and histamine which take part in the allergy reactions.

It is thus seen that these Ig molecules not only perform, but also initiate several important mechanisms, in order to eliminate foreign cells and agents. Below are described the two important mechanisms initiated by Ig molecules. a) The process defined as opsonization 'prepares' the external antigen for ingestion by phagocytes:- There exist special surface Ig molecules termed opsonins . These opsonins trigger the phagocytic process. b) the other important function is the activation of the complement system. [details could be obtained from Lacnmann, P.J., (1979) and Mayer, M.M., (1973)].

2.2. C.3. Other Agents involved

As mentioned, under the section 're-discovery of cellular response', it is now clear that both humoral and cellular agents definitely do bridge and co-operate during various immune reaction in order to step up the total efficiency of the immune system. For example, a group of T cells known as the effector T cells co-operate with B cells to produce specific humoral immune response. The co-operation between the 'B and 'T cells requires an antigen that carries the surface determinants having two combining sites. The effector T cells that co-operate with B cells in the antibody formation are termed as T helper (T_H) cells and are essential for IgG and IgA responses. IgM are T_H independent. But majority of the humoral response are brought forth by thymus independent reactions. Apart from these two situations there exist another group of T cell termed T_S suppressor cells which inhibit antibody production.

T_S are antigen specific supressors, their mechanism of functioning is still unclear. It is also belived that they have a role in 'tolerance'. The third class of T cells are the T_D cells which perform the direct stimulation of local or temporary chronic inflammation. Thus three classes of T cells participates in a immune response in co-operation with the B cells (see Table 3).

Table - 3

Classes of T cells

Group	Class	Function
T_H	T_H	Stimulation of B cells
	T_A	Stimulation of T_C cell differenciation
$T_{C,S}$	T_D	Allergy mediator
	T_C	Lysis of specific cell types
	T_S	Supression of immune response probably tolerance.

The T cells also mediate delayed hypersensitivity by releasing numerous kinds of polypeptides called 'lymphokines'. The lymphokines are said to be mediator agents capable of stimulating the various cells involved in the immune mechanism.

The magnitude and duration of any immune response are limited and are controlled by various mechanisms. Six major responses are thought to be responsible for this control, and they work on the principle of feed-back mechanism.

(i) zLife span of effector cells are controlled and thus requiring a re-stimulation of those effector cells evolved in the immune reaction.

(ii) The antigen removal or decrease in the antigen concentration.

(iii) It is believed that antigen not only stimulate T_H but they also turn on T_S which regulate the synthesis of Ig molecules.

(iv) Steric factors involved resulting in negative feed-back: these are responseble at various levels. a)antigen antibody level b) macrophage immunogen level etc.

(v) It is believed that there is a cooperation of various parameters both of the body and that of the external agent which control the expression of Ig molecules and thus the complete immune reaction.

(vi) It has been recently proposed that, [L.V.Abruzzo (1983)], natural killer cells (NK) terminate antibody response by the elimination of antigen exposed, accessory cells.

With this, we come to the end of an overall view of the immune system (figure 11). The analysis covered as many as possible, but there are many

more issues with respect to the immune mechanism and the system, an insight to these are beyond the scope of this article.

The next topic of discussion is on the structure and function of the Ig molecule, over which a substantial amount of work has been done; following it a description of the Immunoglobulin gene structure, function, expression and organization.

+ HUMAN-IMMUNE SYSTEM +
GENES

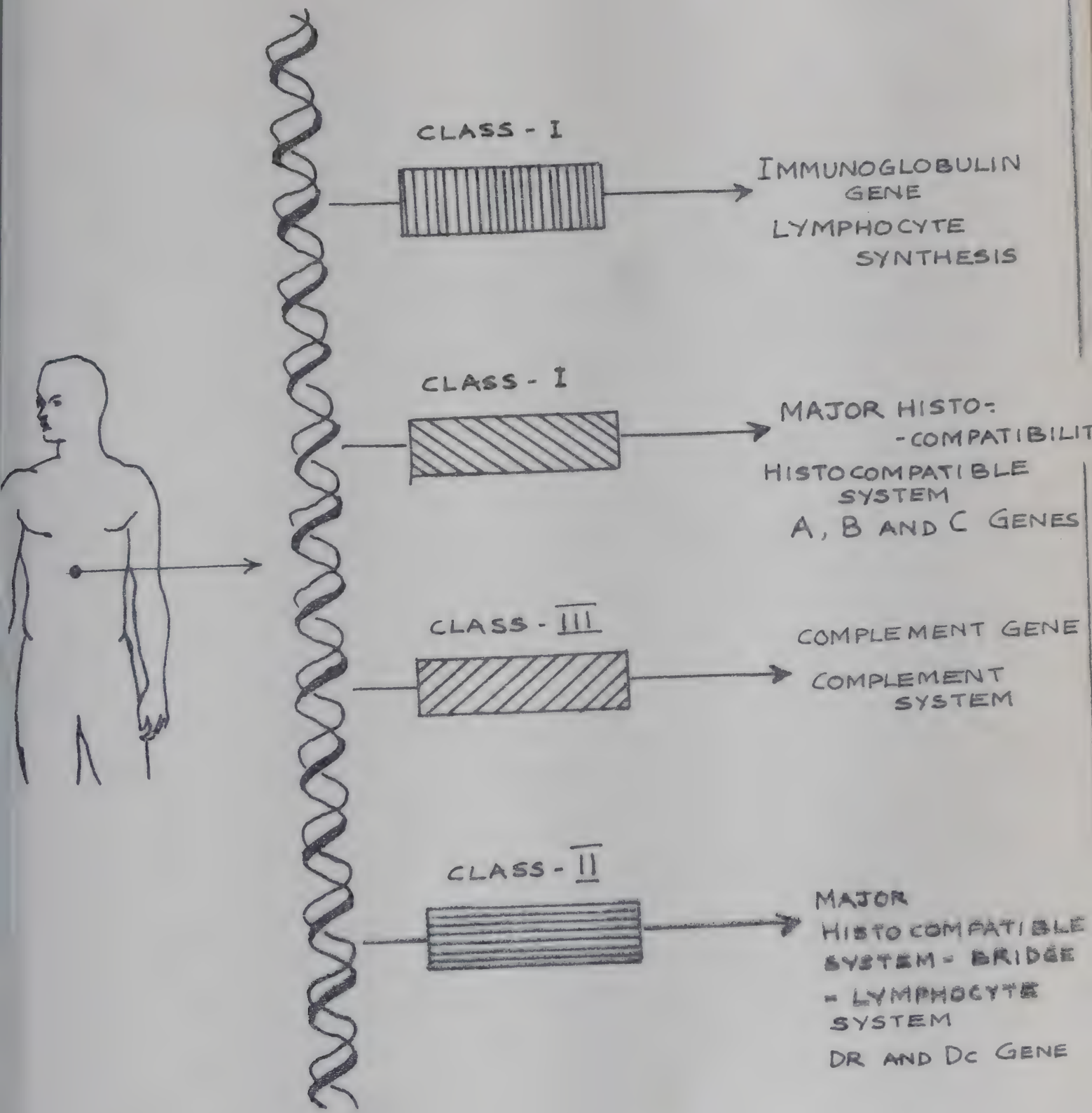


Figure- 11

CHAPTER - 3

IMMUNOGLOBULINS

3.1. Immunoglobulins

Introduction

As mentioned earlier under the topic antibodies (section 2.2.C.2), the best characterized molecules of the immune system are the antibodies . They occur in the globulin fraction of the serum, and thus are termed as 'Immunoglobulins'. These immunoglobulin molecules are evolved to perform two distinct functions.

a) antigen recognition

b) antigen elimination

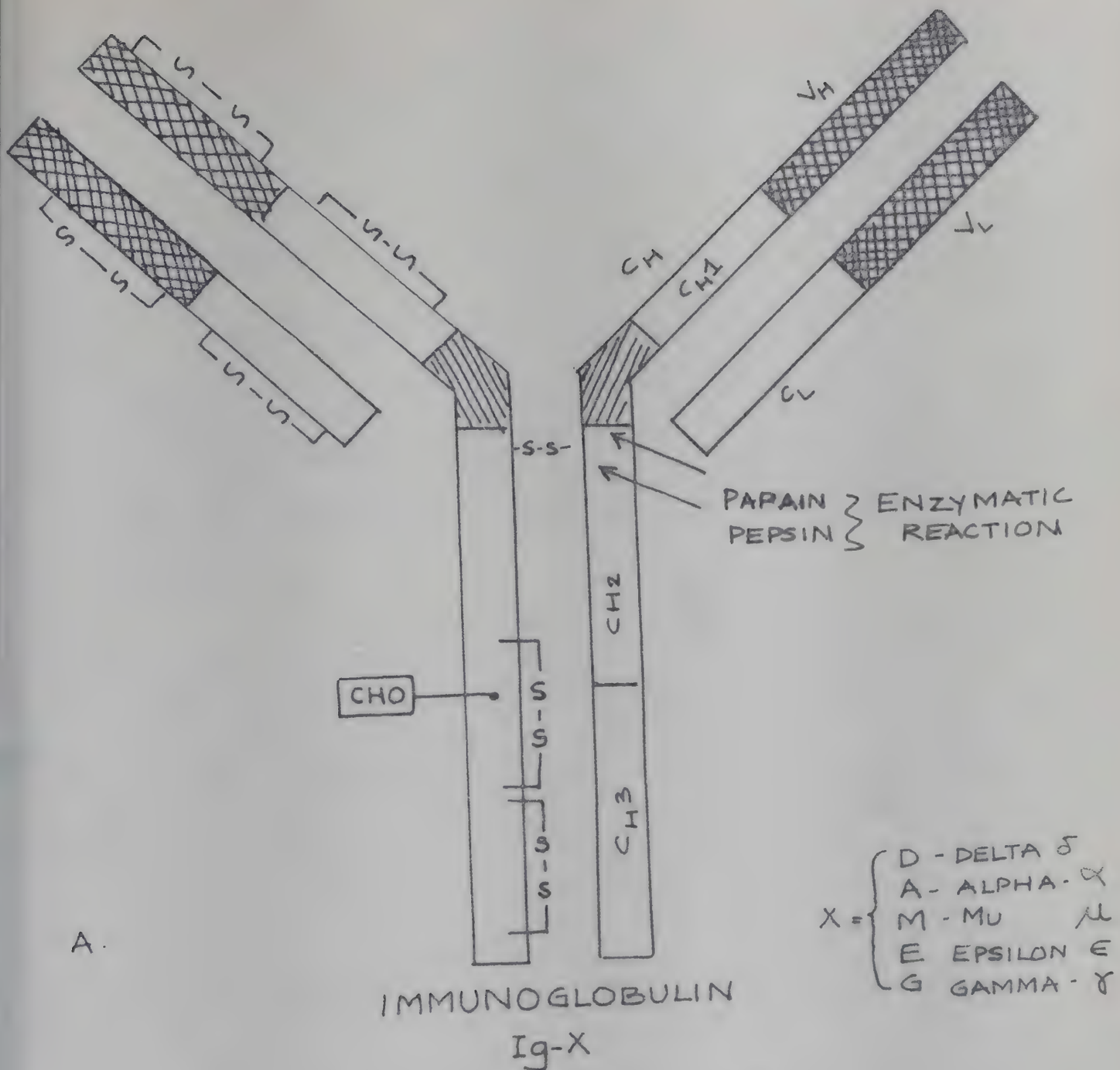
The serum of any vertebrate under normal conditions contain a large variety of proteins, which include five different classes of immunoglobulins. An important feature common to all the five classes, is the fact that the basic structure and the gross chemical properties of these immunoglobulin are very similar. It is therefore, very essential to know these gross chemical similarities in order to understand their functions. In the next few sections, a discussion pertaining to the basic structure of the immunoglobin molecule and organization of their functions based on their structure is done. The study of the immunoglobulin (Ig) molecule is made by taking advantage of abnormal occurrence of homogenous

pathological immunoglobulins production by monoclonal neoplastic lymphocyte cells - a cancer of the antibody producing cells known as 'multiple myeloma'. Myeloma proteins can be obtained in large quantities and can be readily purified to homogeneous molecular species. These proteins have been named after Bence Jones, who had recognized these proteins as a distinct class because of their peculiar thermal behaviour. See Humphrey and Owens (1972) for details. These myeloma proteins were earlier termed as Bence Jones proteins.

The immunoglobulins can be divided into 5 major classes. They are:

a) IgM(mu) b)IgA(alpha) c)IgG(gamma) d)IgD(delta) and e)IgE(epsilon)

A 'typical' immunoglobulin can be represented by an IgG. The molecule is made up of two identical light polypeptides (aminoacid chains)(mol wt.25,000) and two identical heavy polypeptides (mol wt. 55,000), which are linked by interchain covalent bonds - the disulphide bridges. Its typical molecular formulae is $[H_2L_2)_n$ where n depends upon the Ig class (see figure 12A). The L chain of the immunoglobulin molecule, on the basis of antigen specificity can be further divided into two 'families'.


S-S
S-S
S-S
S-S
V_H
C_H
C_{H1}
V_L
C_L
-S-S-
PAPAIN
PEPSIN
ENZYMATIC REACTION
C_{H2}
CHO
S-S
S-S
C_{H3}
A.
IMMUNOGLOBULIN
Ig-X
X = D - DELTA δ
A - ALPHA - α
M - MU μ
E EPSILON ε
G GAMMA - γ

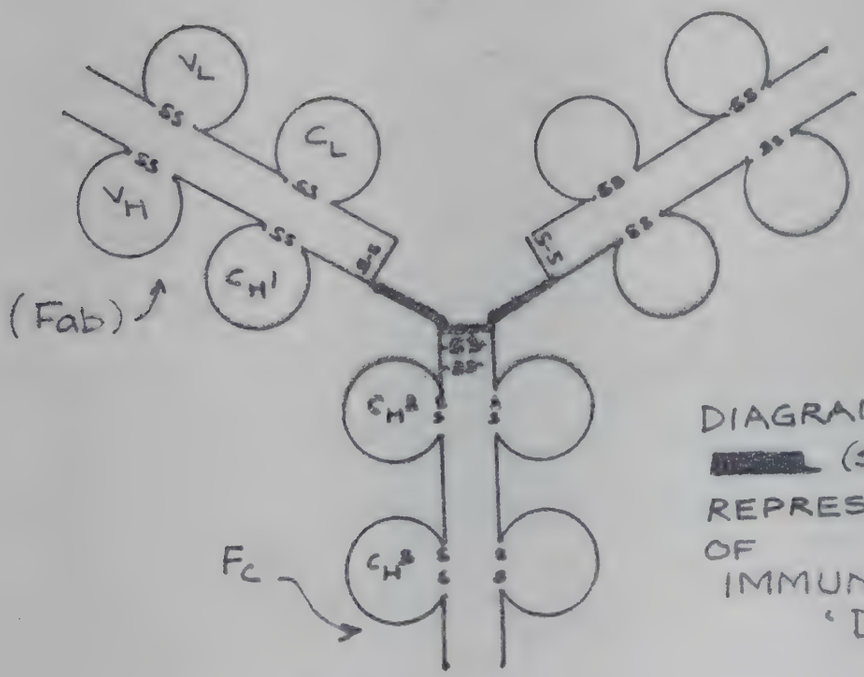

V_L
C_L
V_H
C_{H1}
(Fab)
C_H^2
C_H^3
Fc
B.
DIAGRAMATIC
(SCHEMATIC)
REPRESENTATION
OF
IMMUNOGLOBULIN
'DOMAINS'

Figure - 12 (A & B)

a) The Kappa chain regions(K)

b) The lambda chain region (λ)

Each of these are characterized by an unique sequence at the end of their polypeptide chain. The light chains, K and are found in all the Ig classes, but their H chains are different and are specific to a given class.

True to the fact that the Ig molecules are protein (polypeptide) molecules, they characteristically begin with the NH_2(amino) group and end with a carboxyl(COOH) group. Various 'amino acid analysis' performed on the Ig molecules have revealed the fact that the light and heavy chains could be divided into two parts for convenience. a) Variable region b) Constant region.

A major finding in the determination of the multichain structure of IgG was made by Porter (1959), who found that enzymatic digestion of rabbit IgG produces two kinds of fragments (see figure 12B). They are the Fab (Fragment antigen binding and the FC - (Fragment Constant). On the other hand, controlled digestion by pepsin produced a $F(ab')_2$ which is a fragment having a valency of 2. (Nisonoff et al., 1960) for the specific antigen.

By reduction of the inter chain disulphide, the Fd' fragment or Fab bridge in the H chain [Fab are made up of a L chain] and the Fd: which is different from Fd by an additional H chain are produced.

Amino acid alanysis studies on L and H chain components of the immunoglobulins have shown that these chains certainly and strangely do have unique structural features. When the sequences were analysed it became very clear that the C terminal half were made up of constant amino acid sequences and that of N - terminal a largely variable portion of amino acid residues. Because of possible genetic implication, after a series of analysis it was found that on the basis of the similarities these groups could be further divided into subgroups. Thus three subgroups were proposed for Kappa class and four in case of the lambda class. There exists striking similarities at certain positions and certain amazing differences at other positions in each of the subgroups (WU and Kabat,1970). (see figure 15). When comparative studies were performed on Ig molecules, which were obtained from various species challanged with the same antigen, an important feature emerged out regarding the existance of 'sequence homology' in the amino acid residues at specifically numbered positions (Hill et al., 1966).

Due to the above mentioned fact,four constant 'homology regions' have been defined. They are - C_H1; C_H2; C_H3 in case of heavy chain, and as C_L region on the light chain. In addition to these structural implications resulting in intra disulphide bridges, these region accquire three dimensional tertiary structure which can be considered as typical antigen binding site. Several proposals were made explaining the folding of the IgG molecule in three dimensions (Putnam et al., 1967) and Edelman et al., 1969) and these globular tertiary foldings were termed as 'domains' (see figure 12B). Electron microscopic (EM) studies were then used for understanding the immunoglobulin structure. The first ever direct pictures of the general shape and structure were obtained using this technique. The first such elegant experiment was performed by Valentine and Green (1967). This experiment known as 'affinity labeling experiment' opened the way to the understanding of antigen binding site.

Chemical agents like haptens , showed that certain positions on the polypeptide chain of the Ig molecules covalently link with haptens and these positions varied very much and had no similarities or homologies and thus were termed 'Hyper Variable Region'(hv)

Amino acids making the hyper variable region were tried being named and labelled (Cebra et al., 1971) . It thus supported the hypothesis that the hv region determine the antigen binding site.

An important fact to note is that the E.M. techniques were first used for structural studies, following this was the x-ray crystallogeaphic technique. This technique found its mark in determining the finer aspect of the structure of the immunoglobulin molecule.

A detailed analysis of the structural aspects of the immunoglobulin molecules are discussed below.

3.2. The Immunoglobulin chains

3.2.1. Light chain: types and sub types

As mentioned earlier there are two types of light chain regions which are defined on the basis of their multiple structural positions a) the Kappa light chain region b) the lambda light chain region.

Although they are molecules of apparently similar function, the sequence similarities between the two types are just about 40 percent . Analysis show that there exist far greater homology between human and mouse Kappa chains suggesting a strong evolutionary

link up. The proportion of Kappa to lambda varies between species with the Kappa: lambda ratio being 2:1 in man. There are three subgroups with respect to K chain . Lambda chain has been divided into four subgroup which are products of genetic duplication. These subtypes have characteristic amino acid substitution at various positions.

Table - IV

Critical amino acid positions

C n	112	114	153	163	190
C 1	Ala	Ser	Ser	Thr	Lys
C 2	Ala	Ser	Ghy	Thr	Arg
C 3	Ala	Ser	Ser	Thr	Arg
C 4	Ala	Thr	Ser	Lys	Lys

The pattern of substitution suggests that the C after mutation and duplication gave raise to other groups (Adapted from Fett and Deutsch (1975)).

3.2.2. Heavy chain: types and subtypes

There are five distinct heavy chain classes $\mu,\gamma,\alpha,\delta,\varepsilon$. The heavy chain determines the immunoglobulin class and each combines with a specific Kappa or lambda light chain.

Table - V

Properties of immunoglobulin classes

Class	Mol wt.	Domain	Molecule	$[H_2L_2]_n$
			M.W.	n
IgM μ	70,000	5	900,000	5
IgG γ	50,000	4	150,000	1
IgA α	55,000	4	300,000	2
IgD δ	65,000	4	180,000	1
IgE ε	65,000	5	180,000	1

In many species there, are several versions of both the gamma and alpha heavy chain classes. In man, sequence studies have shown 95 percent homology between different subclasses compared to 45 percent homology between classes. (structural differences adapted from J. Gally, in the Antigen. M. Sela (ed.) A.P. N.Y. 1973 p.203).

The basic as well as the gross differences between immunoglobulin classes and subclasses are seen in the

number of domains, the arrangement or position of disulphide bridges and over all percent of carbhodydrate. (from Secher 1979).

The differences are schematically represented. There are three constant region domain in γ and α (C_H, C_H2, C_H3) four in μ and ε. See next section for details. (see figure 13A).

3.3. Immunoglobulin structure - secondary and tertiary aspects

In the earlier section, the primary structure of the immunoglobulin molecules was discussed. Among the various techniques used for these studies, amino acid sequence analysis was the most important.

In order to understand the secondary and the 2D and 3D structure of the immunoglobulin molecules, two techniques have been used.

a) Electron microscopic technique

b) X-ray crystallography

The secondary and the 3D structure analysis would help us in understanding the biological implication of the immunoglobulin molecules which basically include the antigen binding and the complement activation. The electron microscopic technique was used in the early 1960's

and gave way to the X-ray crystallography due to its higher resolution capacity in the 1970's. The two techniques were made best use of in order to get the detail analysis of the structure.

3.3.1. Electron microscopy (EM)

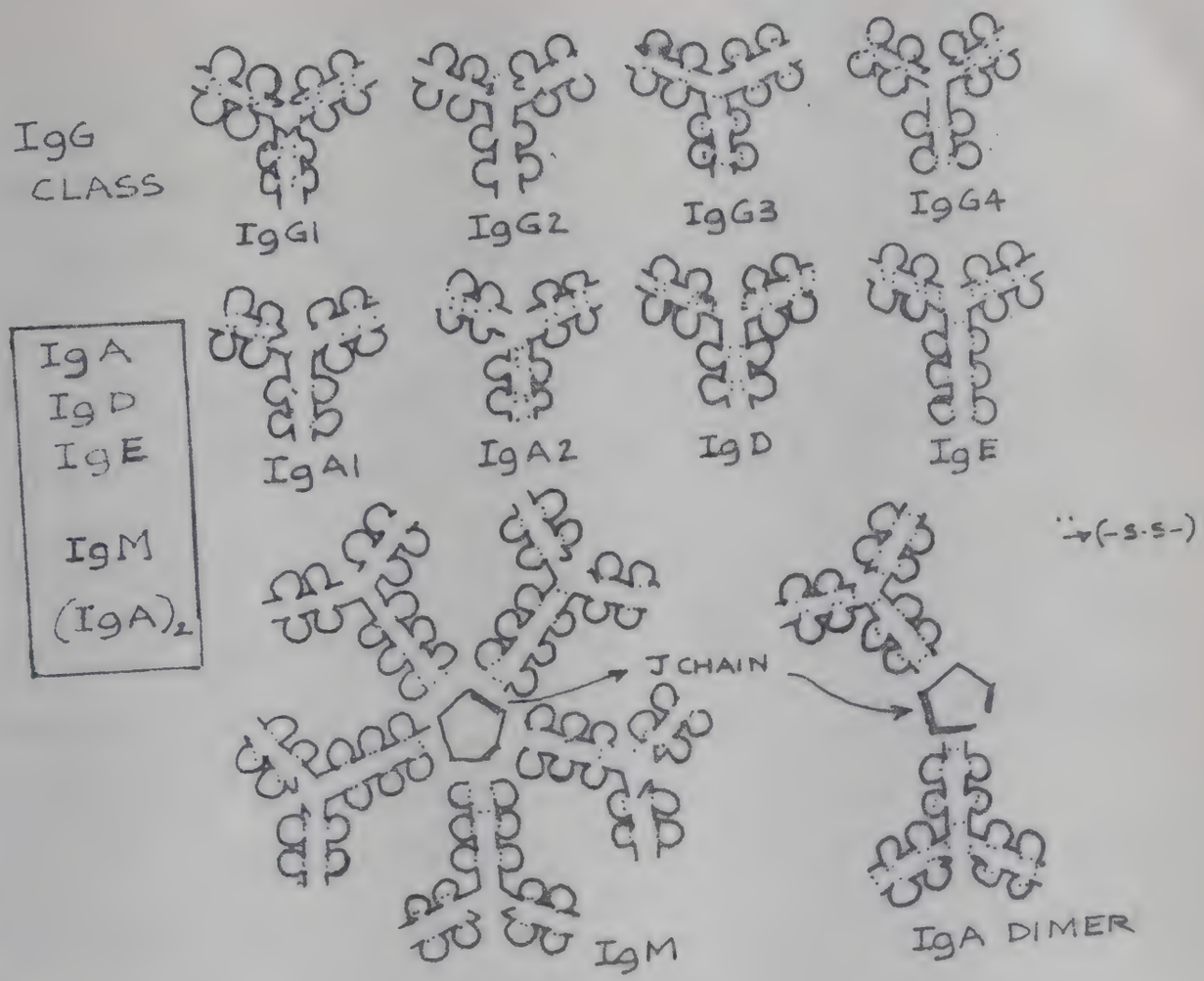
Studies of the structure of Ig using EM technique were done on antigen-antibody complexes using negative staining.

The earliest electron micrographs of Igs were of virus particles linked by Igs. This was followed by using ferritin IgG anti - Ferritin complexes under specific conditions . This study gave the first picture of a typical immunoglobulin which was shaped with a variable angle between the arms. $F\gamma ab)_2$ was also found to have the structure of with its stem missing.

Valentine and Green (1967) first introduced the much appreciated bifunctional c hapten as the 'antigen technique'. Two or more IgG molecules linked together by the hapten via their combining sites were seen under the staining. The advantage of the system was the fact that resolution and contrast were much better as compared to the situation when a bulky antigen was made to combine with an Ig and then stained. The studies by Green and

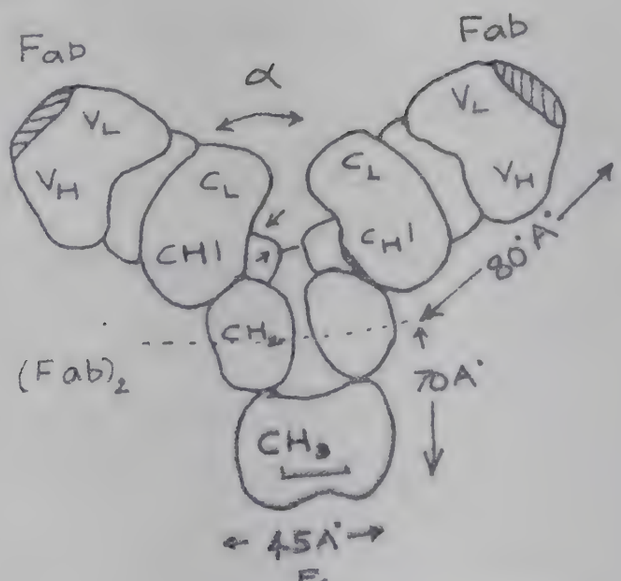
IMMUNOGLOBULINS

A.



B.

IMMUNOGLOBULIN MOLECULE →



A SCHEMATIC DIAGRAM OF Ig-G VARIOUS DOMAINS REPRESENTED

Figure - 13 (A&B)

Valentine illustrated two very important points (see figure 14E).

a) The antigen combining site is on the end surface of the Fab arm.

b) In Ig ,there exists the freedom of movement of Fab arms with respect to each other and the Fc portion.

Analysis of the structure of IgM were done using the above technique. IgM being a pentamer was detected and was described as a characteristic 'star-fish' like molecule . It was seen that the IgM takes many different configurations. Detailed E.micrograph of IgM were taken by Fein stein et al.,(1971) . Analysis were also done on IgA by the same group.

3.3.2. X-ray crystallography

In the E.M. technique,individual molecules are photographed and can be studied individually or separately. X-ray technique permits a much greater scope by allowing a far greater resolution by integrating the common features of the molecules held in a regular crystalline array. The information obtained from the crystals of whole Ig molecule confirm and extend the information from the EM.

An extension analysis on the X-ray techniques used are given in cold spring Hartor Symposium 1971 and Matthews 1976 In short here is what the X-ray crystallographic technique is used as.

X-ray's are capable of being diffracted by solid or crystalline objects. The diffraction at the order of inter atomic distances is made possible due to the fact that the wavelength of X-rays used are of the order of the inter atomic distances in a lattice. The diffracted pattern obtained on a photographic film project the position of the various atoms (which can be calculated out by using mathematical formulae) in three dimensions.

A crystallographic analysis of a whole IgG molecule was-first reported by Sarma et al., (1971). The studies indicated two fold axis of symmetry that runs through the inter H chain disulphide bonds. Electron density maps (techniques of X-ray analysis) indicated two globular regions which were later interpreted as F_C part of the molecular where the two C_H2 and C_H3 domains are related by an exact two fold symmetry (Gold stein et al., 1968) The other globular structure corresponded to the Fab part of the molecule. Four different structures of IgG were proposed based on the data obtained. In one of them, the

the IgG molecule was considered to have a shape in the form of a 'T' , however, an EM study of the same substance (Labaw and Davies,1971) showed a shape molecule. It was assumed that a lattice force may induce a T shape in this particular crystal form, a shape that might get changed under different conditions to different forms.

The other structures proposed could be ignored because it was shown by Pilz et al. (1970) that the Ig molecule in solution took to a T form and taking this as an essential criteria any other configuration would not be possible as it would result in unstable situation.

3.4. Three dimensional structures of the light and heavy chain regions

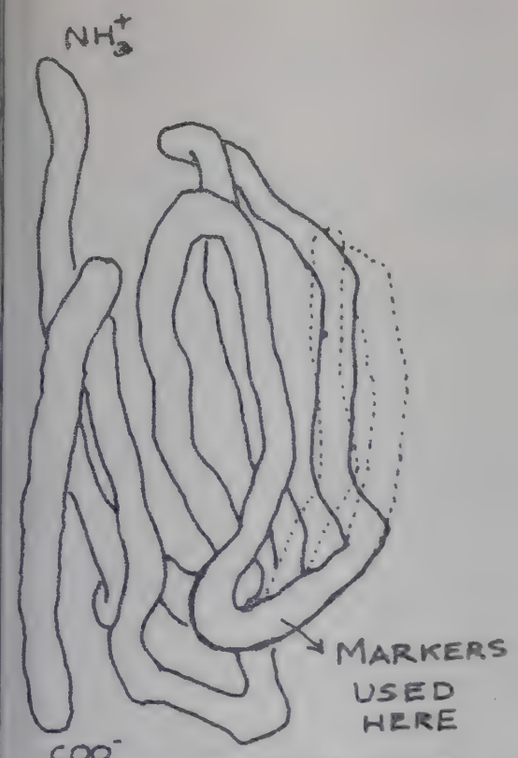
Detailed X-ray analysis have been performed on light chains: (Schiffer et al., 1973 Edmundson et al., 1974) lambda and Kappa (Epp et al., 1974) chains. In addition to the X-ray analysis performed on light chain crystals, analysis on Fab crystals have also thrown light on the structural configuration on these L chains.

Analysis by Edmundson gave interesting results. There were four intrachain disulphide bridges, the V and C regions were well expressed along with the linking region.

Light region was divided into two parts, the two globular domains and the V and C domains (Soloman and McLaughlin ,1969). As mentioned earlier, although the two chains are to perform similar functions the two differ a lot in 3-D structure, in one of the chains the major area axes of the V and C region make an angle of 110°; (see figure 13B) in the other angle of 70°. The V and C region resemble each other in shape and in polypeptide-chain folding. Residues 48-60 which include 'hypervariable region', have no equivalent C domain. The structure of a crystalline dimer of the variable region (Kappa) of the mycloma protein, showed that 50 percent of all amino acid residue are located in one of the two βsheets present in a V Kappa monomer. From analysis (both X-ray and aminoacid sequence) it was found there exist three hypervariable regions in the light chain region.

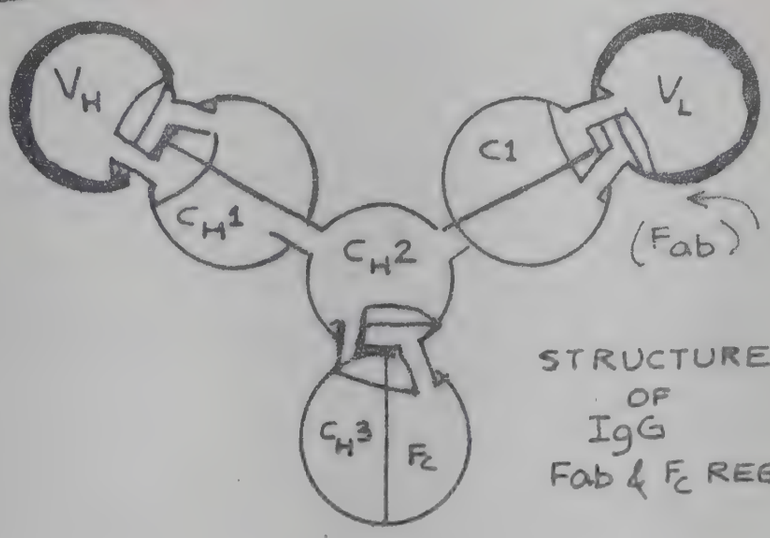
A detailed study of Fab (Poljak et al., 1972) showed that the Fab fragment consisted of two discrete globular domains , t'he V abd C containing V_L and C_H, and C_L and C_H1 regions respectively (see figure 14B).

It can be seen that as against studying an immunoglobulin molecule based on the regions that have been defined i.e. Light chain and heavy chain and



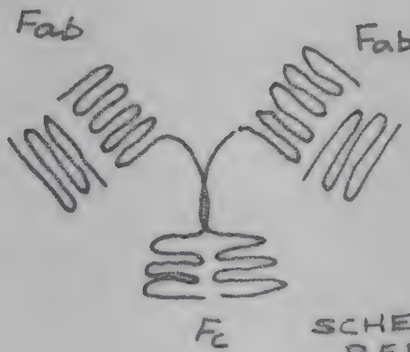
IMMUNOGLOBULIN FOLD

B.

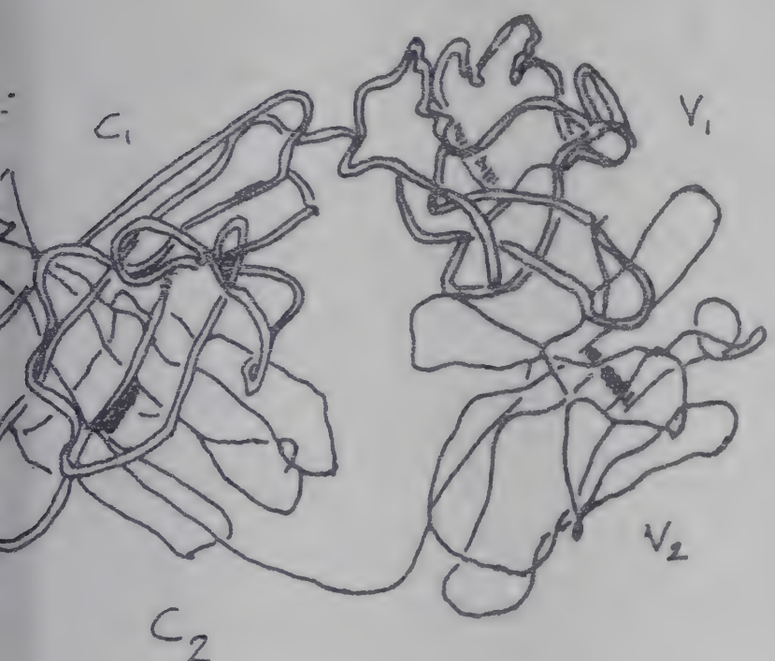


STRUCTURE OF IgG Fab & F_c REGIONS

D.

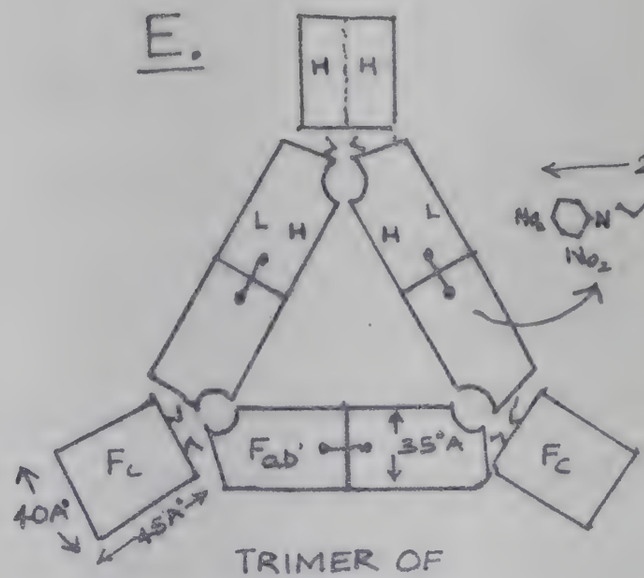


SCHEMATIC REPRESENTATIO OF Ig-G PROPOSED BY NOELKAN



3-D - DIAGRAM OF Mcgλ-chain BENCE-JONES DIMER 3.5-A° RESOLUTION

E.



TRIMER OF ANTI-DNP RABBIT IgG IMMUNOGLOBULIN

Figure- 14 (A, B, C, D & E)

further, the constant and variable regions, the crystallographers could study Ig molecules only in fragments such as (a) Fab (b) Fab' (c) $F(ab')_2$ and combined antigen antibody site. The various domains are a result of the structural studies on the different fragments.

A low resolution analysis of Fab' (Poljak et al., 1972) show that four tetrahydral arrangements of the domains, two from the light chains and the other two from the heavy chains (V_H and C_H1).

The structure obtained from this study of Fab' was extended for the explaination of the structure IgG. In the representation as shown in diagram, the V region is made up of V_L and V_H and the C region. C_1 is made up of C_H1 and C_L, C_2 and C_3 are made up of $(C_H2)_2$ and $(C_H3)_2$ respectively. The C2 and C3 regions are responsible for the two fold symmetry of the heavy chain.

A higher resolution analysis have also supported the above conclusion. (Poljak et al., 1973 and 1974). The analysis show that C_L - C_H1 interact not only much more than expected but also do so more than V_L-V_H subunits The C_L, C_H1, V_L and V_H subunits are very much similar in their three dimensional folding. Although V_L and the V_H share the basic immunoglobulin fold of the C_L and C_H

(see figure 14A) they include an additional length of polypeptide chain in the form of a loop not present in C_L and C_H1. In the L( ) chain of the IgG, a deletion of seven amino acid residues in V_L results in a shortening of the additional loop of the polypeptide chain, making the V_L structure of IgG more similar to that of the C_L and C_H1 subunits than to V_H.

In short below are given the essential aspect of Ig structure as obtained by 3-D analysis. The Fab consists of four tetrahedrally arranged globular units which orient themselves in 3 dimension (see figure 14C) And also containing is the amino acid sequence of a homologus region V_L and V_H much form a globular domain (V), C_L and C_H1 another of globular domain (C). The C_L and C_H1 homologous regions consists of a β sheet made up of four antiparallel strands of polypeptide chain and another β sheet containing three antiparallel stands. 50 percent of amino acid residues are folded in β pleated sheets. Absence of helkal structure is very conspicous in this region.

Thus the description of the typical Immunoglobulin molecule shows its complexity.

With this, the discussion on general structure of an immunoglobulin molecule occuring under normal circumstances is included. Before analysing individual immunoglobulin family, a brief description about the antibody combining sites are mentioned.

The hypervariable sequences have been recognized by statistical analysis of L chains(Wu and Kabat 1970; Kabat and Wu 1971) around position 30, 50 and 95. A similar analysis was performed on the H chain. Initially,the hypervariable region in case of H chain was less definitive (Kabat and Wu 1971). But later analysis by Cebra et al(1974) indicated three hyper variable residues at positions 25-30, 55-65 and 100-110. The hyper variable regions of the immunoglobulins were postulated to specify the conformation of antigen binding site. One more position 81-85 was debited in case of H chain, but was not involved in the antigen binding site Support to all these hyper variable positions and that of antigen binding site positions were confirmed by using (Singer et al., 1971, and Givol et al., 1971) at finitely labelling techniques where residues V_H and V_L sequences coincident or adjacent to hypervariable positions were

detected by the labbled reactive haptens. Cross linking of H and L chains by affinity labels (Girol et al., 1971) indicated that H and L regions occur in close proximity to the antigen binding site. (see figure 15).

Thus it is seen that the antigen binding site constitutes one of the most essential part of the Ig molecule.

3.5. Immunoglobulin classes:

A discussion on the 5 different classes of the immunoglobulins.

3.5.1. Immunoglobulin 'Gamma' : IgG

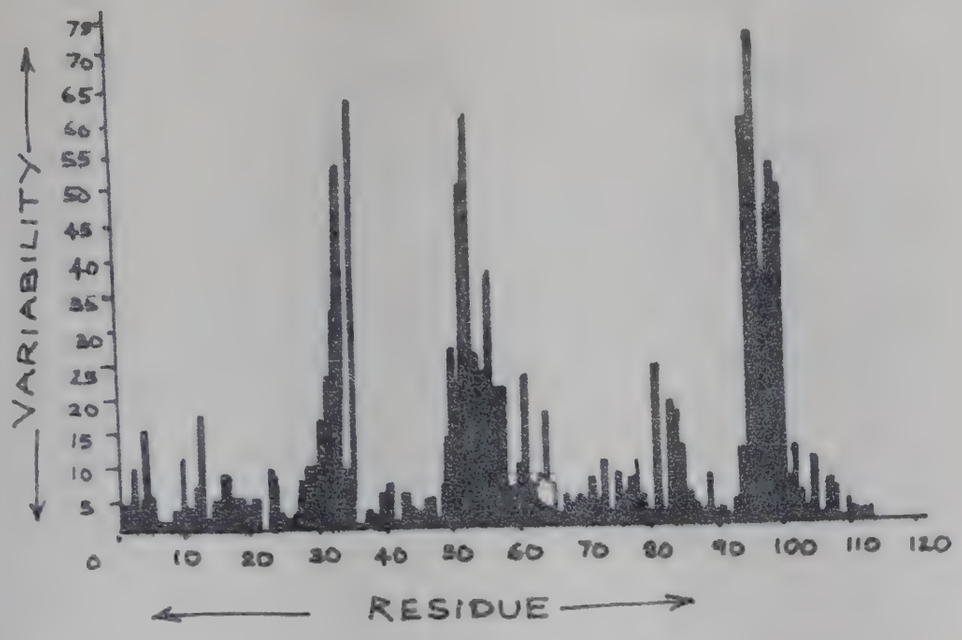
The four polypeptide chains of IgG are folded to give three large substructures two of which denoted Fab, are identical and possesses an antigen combining site and the other the F_C region.

Noelken et al., (1965) proposed first the molecules configuration. Later models confirmed the structure proposed by Noelken and co-workers. (See figure 14D).

IgG is the most abundant of the immunoglobulins. In the serum, its concentration reaches a very high levels. A schematic diagram of IgG can be divided into a Fab and F_C fragments; each of these fragments

A.

HEAVY CHAIN



B.

LIGHT CHAIN - (K)

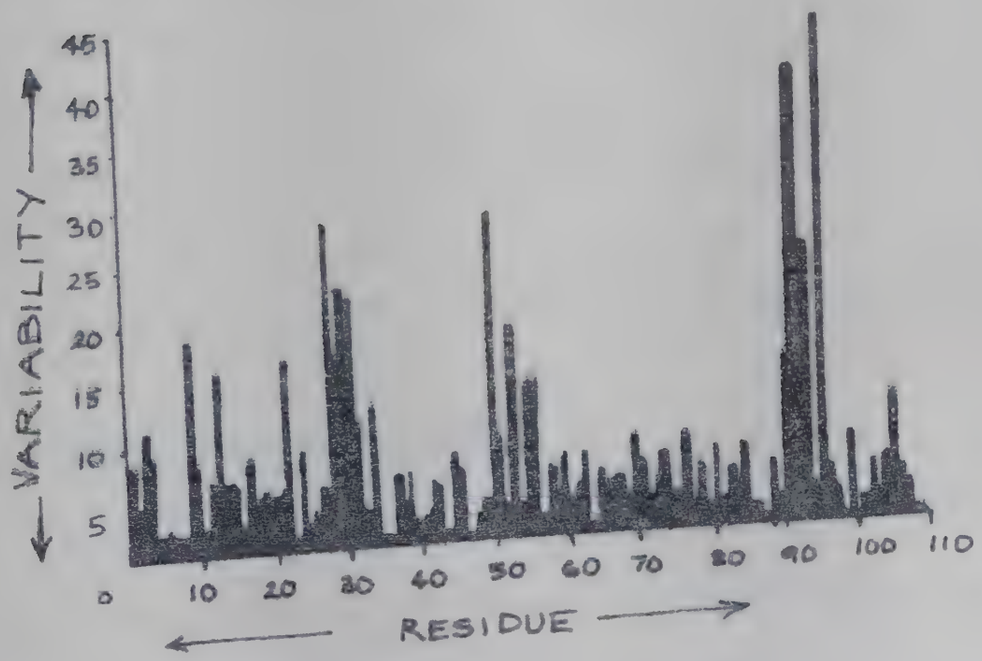


Figure - 15 (A&B)

comprises various domains. Fab consists of the variable region of light V_L chains and that of heavy V_H chains. The shapes of these domains were given by Poljak 1973; Segal et al., (1974). F_C consists of the second C_H2 domains and third (C_H3), C regions of the H chains. Two antigen binding sites are located at the outer tips of the Fab and are shown in the diagram. The binding of the complement proteins are said to occur in the C_H2 domain.

The IgG molecule is said to be capable of taking part in various immune functions. It has a long half life (23 days). Their complement activation are said to occur in the placenta. This class of Ig molecules have a direct hand in the control of the blood borne dissemination inclusive of bacteria, parasites, and fungi Receptors of IgG exists on human monocyte and also few lymphocytes. IgG also activates macrophage ingestion of eliminated particles. The average affinity for antigen of the IgG produced in the primary immune response increases in with time after immunization.

3.5.2. Immunoglobulin Mu

Right since the initial observation by Heidelberger and Pedersen (1937) of macro globulin antibody against bacteria in horse, immunoglobulin M (IgM) has been

recognized as a major class of Ig molecules in human's also. In the serum the IgM is said to occur as a pentamer, each unit of the pentamer said to be made up of two L and two H(μ) chains which are held together by both non-coralent forces and by disulphide bridge (reviewed Metzger, 1970). A third unrelated chain known as the J chain is also found present in the IgM and its presence was explained by Koshland(1975). The overall molecular weight of the pentamer is ablut 8 900,000 (Dorrisgton and Tantord, 1970) with ten potential antigen binding sites (Reisin et al., 1975). It can be seen that the IgM molecule contains five homology domains (V_H and $C_\mu 1$ to $C_\mu 4$) (Wantanabe et al., 1973) . The disulphide bridges that link the IgMs subunits are reported to be in the amino acid residue position no.414 (Beale and Feinstein 1969). The conformation of IgM in solution have been studied in far less detail. This is due to the fact that most studies were done on human macroglobulins.(for secondary and 3-D structure in solution (see Ghose,1971).

IgM due to its large size is restricted entirely to the intra vascular space . These molecules are found to be highly effective and efficient agglutinators of bacteria and RBC and the complement activity is said to be very strong. This Ig molecule has its importance

in the primary immune response as their percentage of concentration in serum is maximum compared to the other Ig classes.
Because of their size, they do not enter the Placenta. They are displayed as monomers on the surface of B cells.

3.5.3 Immunoglobulin (alpha) IgA

IgA molecules is said to occur in several polymeric forms. In serum, under normal conditions they are in their monomeric state, but a dimeric state or high oligomer also occurs. Polymeric forms includes the J chain (Koshland, 1975). It occurs with an additional component known as secretory component when secreted by mucous membrane. Secretory IgA is not a dimer but two monomers sandwitched (Svehag et al., 1970).

The evidence of globular domains in IgA was first seen in EM (by Green et al., 1971) and later confirmed by X-ray analysis (segal et al., 1974). It is seen that IgA has a lot of similarities to that of IgG (Segal et al., 1974).

Although IgA is the second most abundant immunoglobulin its importance as mentioned earlier lies with its secrectory function. They are produced in large concentration in the

gastrointestinal, tonsils and other cavaties. The activation of the complement is not by the classical pathway because it does not cross placentra due to structural features. They are also found in the milk saliva, sweat and tears etc.

3.5.4. Immunoglobulin (delta):

This Ig molecule is found in the serum in a very low concentration (Leslie et al., 1975). The role of IgD has been puzzling though evidence have been mounting on the hypothetical suggestion that IgD may be present to act on the chronically present antigens (Lertora et al., 1975). IgD complement fixation is also not by the conventional manner but by other pathways (Konno et al., 1975) IgD is said to be a surface immunoglobulin with a large concentration of them occuring on the surfaces of lymphocytes.(Van Boxel etl. al., (1972)). Strange enough all the IgD light chain are lambda chains.

The IgD molecule has its structure very much similar to that of IgG: two heavy delta (H delta)

and two light chain (mainly). A single disulphide connects the delta chains (Spugelberg et al, 1970). The molecular weight is reported to be somewhere around 60,000(Leslie et al., 1975) and 50,000 (Goyert et al., 1976) . The higher molecular weight can be accounted

for as an additional globular fraction attached to the stem.

This class of Ig's were incidently discovered in the mid 1960's by Rowe and Fahey, when they encountered a myeloma protein totally different to the then known Ig classes. The actual immune activity is not known but is believed to be in recognition.

3.5.5. Immunoglobulin E (Epsilon)

IgE represents one of the most important and essential of Ig molecules as they play a very strange role-mediation of allergy antigens, of any specific immune response. They are found in the gastric, lymphatic node and respiratory sectors of the organism, but strangely in low concentration in the serum (see review by Ishizaka 1970) . They are found associated along with basophils (allergy reaction and histamine relaasing factors), and appear to be involved in the activation of various cells thus indirectly responsible for releasing various chemical substances. (Ishizaka ,1972 and Kay and Austen ,1971) . Much of the understanding has so far been due to the analysis of IgE myeloma (Johansson et al., 1967; Nezlin et al., 1973).

The IgE molecules consists of two light and the H(eplison) chains linked by inter disulphide bridges with molecule weight being about 188,100. There exists two intra disulphide bridegs. The deficiency in IgE have resulted in undue susceptibility to infection.

3.5. Sub-class of immunoglobulin molecule

Thus the various Ig classes have been described. There exists even subclasses of Ig link IgG is made up of actually IgG1, IgG2, IgG3, and IgG4 each differing from the other with either addition or deletion of amino acid. sequences at unimportant junctions. IgA is made up of IgA1 and IgA2 and similarly IgD of IgD1 and IgD2. There are minor differences in effector functions of these subclasses.

3.6. Biological activities of Immunoglobulins

As mentioned earlier, most of the biologically directed functions, other than antigen binding performed by $(Fab)_2$, are performed by the F_C region. This region is claimed to play a part in the movement of IgG across various membrane. The placental transfer is dependent on Ig subclass. Like any conventional proteins,

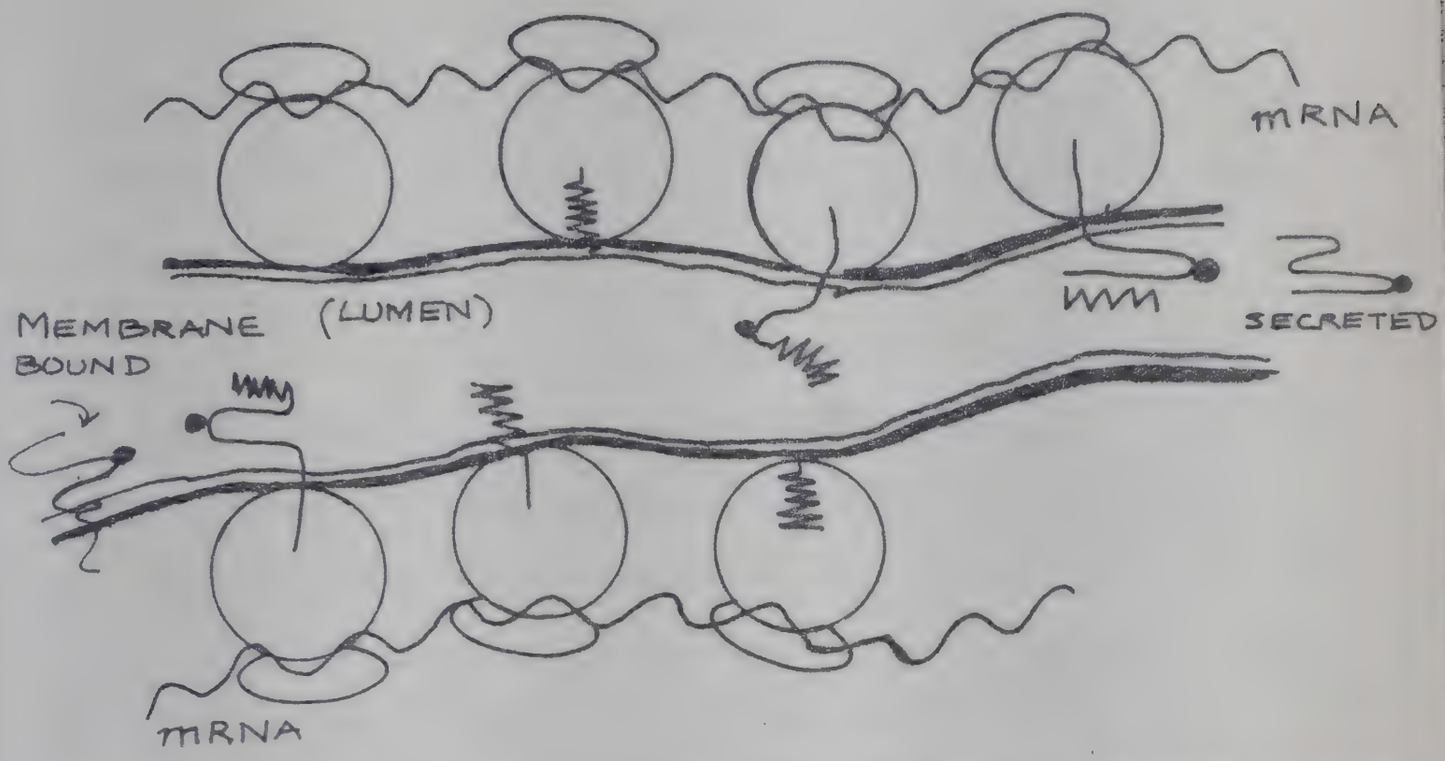
Figure : 16-A : A diagramatic representation of the synthesis of heavy chains of secreted and membrane immunoglobulin. Once the leader or signal sequence has been synthesized the ribosomes bind to the endoplasmic reticulum (ER) and the nascent chain passes through the membrane.

For a secreted protein the C-terminal end of the chain passes light through the membrane of the ER and it is then thought to be secreted from the cell without having to cross another membrane . For certain membrane proteins the completed chain remains embedded in the membrane with C-terminal on the inside of the cell, represents carbohydrate, leader sequence hydro phobic residues.

Figure : 16-B : Shows the formation of secretary. IgA . The IgA dimers are produced initially as monomers but secreted later from plasma cell as dimers with the linking J chain. As the dimer passes through the epithelial cells they acquire a secretory component and enter the body lumen as secretory IgA molecule.

Figure - 16 (A&B)

A. SECRETED & MEMBRANE-Ig



+ B. SECRETORY IMMUNOGLOBULIN → IgA DIMER

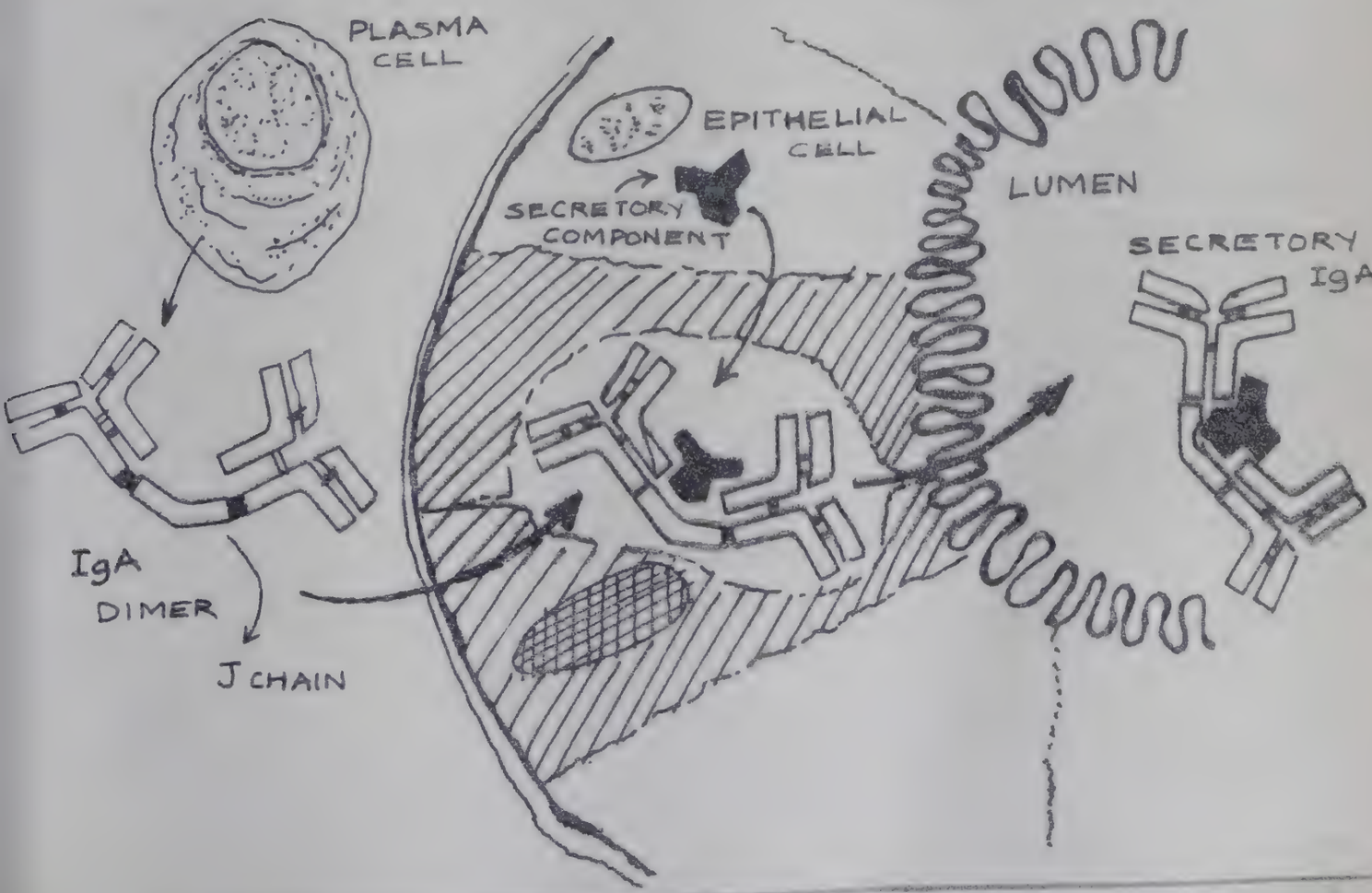


Figure - 16 (A&B)

immunoglobulins are synthesized by ribosomes of the endoplasmic reticulum (see figure 16 A and B) . It is claimed that the chains are synthesized separately. Fetailed analysis of the immunoglobulin synthesis at the ribosome level have been done. (by Milstein et al., 1972; Blobel et al., 1975).

Under each Ig class, Ig are not only found in serum but are also found associated with various tissues. The Ig get themselves incorporated to the membrane and become transmembrane protein.(Vitetta et al., 1971; Ratt et al., 1973; Petnis et al., 1974; Fu et al., 1975). One could obviously expect that in addition to the antigen binding founction, the effector functions are performed by the Igs in order to make their reactivity much more efficient and meaningful. Recent evidence suggest that there are some differences between the 'free serum Ig's' and 'surface integrated Igs'. (Williams et al., 1978).

CHAPTER - 4

IMMUNOGLOBULIN GENES

4.1. Immunoglobulin gene : An introduction

The genome or the genetic apparatus of any organism, are the set of instructions which are inherited from the parents of the organism, and are carefully preserved and propagated. Under normal conditions, the genome amounts to just over a million genes (the Deoxyribo-nucleic acid molecule-DNA) and the genes carry the blue prints of all functions of the organism.

The genetic information encoded in the genes is transferred to the proteins which are mainly responsible for the metabolic reactions of the organism. Structurally, they are made up of amino acid molecules and the synthesis of these protein molecules is under the direct control of DNA molecule. They are encoded in such a manner that the sequence of nucleotides which makes 'sense' by carrying information, form the '<u>gene</u>' and each complete information or 'sense message' corresponds to a polypeptide or a protein chain. This is the essence of 'one gene one polypeptide' theory. [Beadle and Tatum, 1941).

But Immunoglobulin molecules are a very unusual family of proteins. In any individual, there exists the capacity of that individual to generate virtually unlimited numbers of not only the same but different immunoglobulin molecules which recognize and bind to many millions of non-self alien particles. This only implies that there must exist many million genes, immunoglobulin genes - alone. Yet as mentioned earlier the genome amounts to just over a million genes! The paradox as it seems apparent is how limitless immunoglobulins are produced when there are limited number of genes? This happens to be one of the most debatable and puzzling issue before the immunologist!

As discussed earlier, the most intriguing aspect of the immunoglobulins are their chemical composition - they are made up of two chain of amino acid molecules. The two chains, the heavy and the light chains are essentially the same as far as the amino acid sequences are considered, except at a few regions wherein the sequence or residue are different. Based on these facts, the immunoglobulin molecules have been divided into two regions a) the constant region b) the variable region. The essense of similarity between Ig molecules

lies in the fact that different V regions are joined to the same C region . Thus,unlimited Ig molecules with different V regions have been associated with the same C region. This is a very unusual property of protein molecules! Becoming aware of this unusual property of immunoglobulins has been a recent development, compared to the classical/ one gene one polypeptide nature of protein, (Beadle and Tatum, 1941) which explains the production of many biologically conventional proteins. To explain these, William Dreyer and Claude Bennett in the year 1965 proposed the 'two gene one polypeptide hypothesis' on the basis of the chemical data present on K-chain reported then. Almost at the same time, two different labs independently showed that the Kappa chain could be divided into a variable and constant region,after analysing myeloma protein. (Hclschmann and Craig 1965; Titani and Putman, ,1965).

This hypothesis was based on the fact that if the immunoglobulin chain, 50 % variable and the remaining constant region were coded in a single gene then, a mechanism must have evolved to preserve the unchanged constant sequence from being mutated. But at the same time allowing the variable region to get

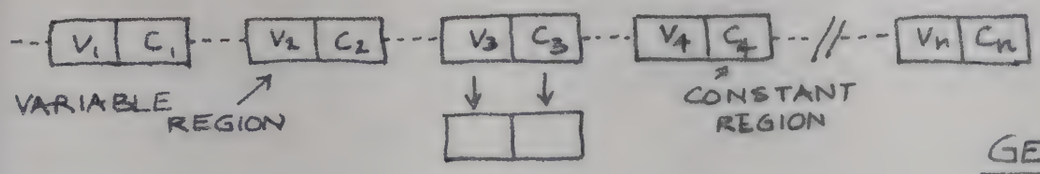
randomly mutated. This seemed highly impossible and unlikely biologically. Thus, instead of assuming the classical/one gene one polypeptide theory, the new mechanism was proposed. Dreyer and Bennett also proposed that there are several separately encoded variable region genes in the DNA of germ cells, but only one constant region gene. If there exists only one constant region, a mutation in this region will result in a changed constant aminoacid sequence in every antibody molecule. Also implicit in the proposal was the idea that the separate genetic information must some how come together to form a continuous genetic information. This proposal initially came under strong attack, but now has formed the central tenet of molecular immunology (see figure 17 - 1 and 2)

The existance of separate V region and C region genes recently has been demonstrated in two different experiment with nucleic acids from mouse cells.

Susumu Tonegawa and Nobumichi Hozumi, then at Basal Institute for Immunology, performed experiments with embryonic DNA. They cleaved the DNA into small fragments by digestion with restriction enzymes. They suggested that the V and C regions of the K-light chain are coded by two separate genes in the germ line.

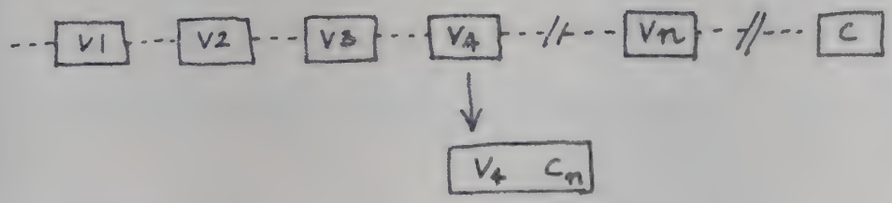
Figure - 17 (0.1, 0.2, 0.3, 0.4A & 0.4B)

ARRANGEMENT OF THE IMMUNOGLOBULIN GENE +



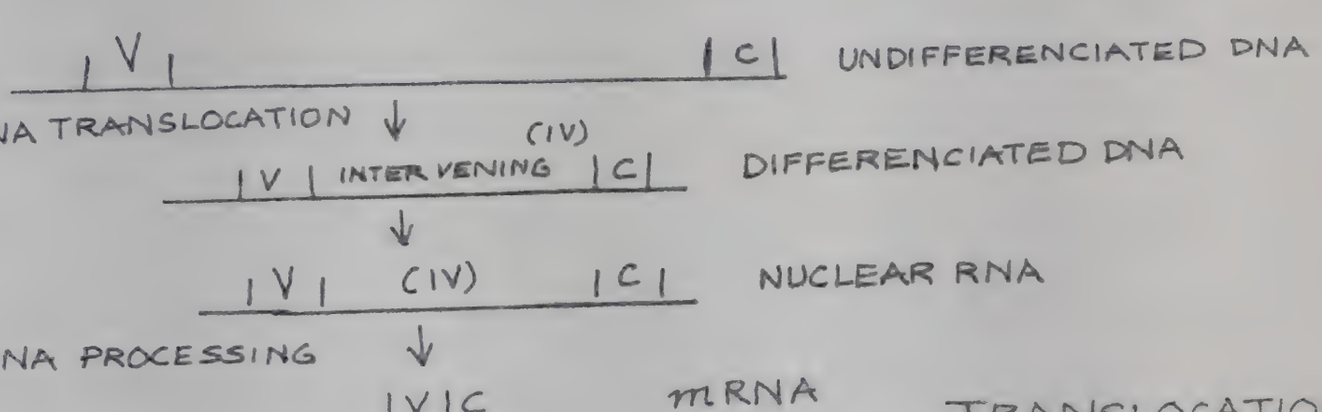
GERM-LINE MODEL

2.



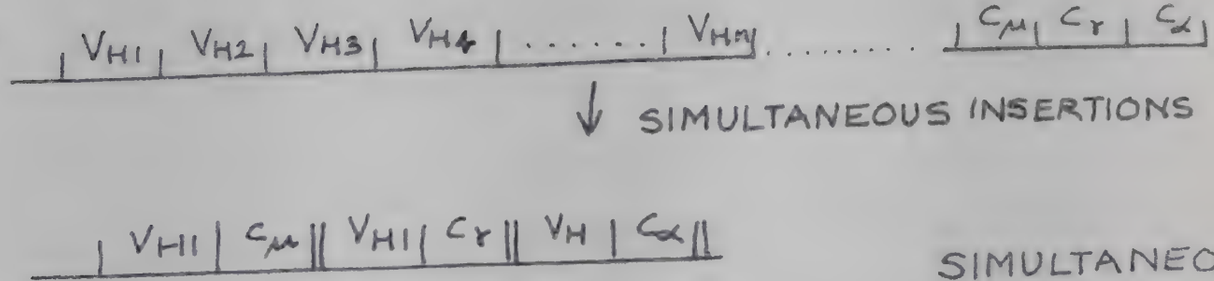
RECOMBINATION MODEL

3.

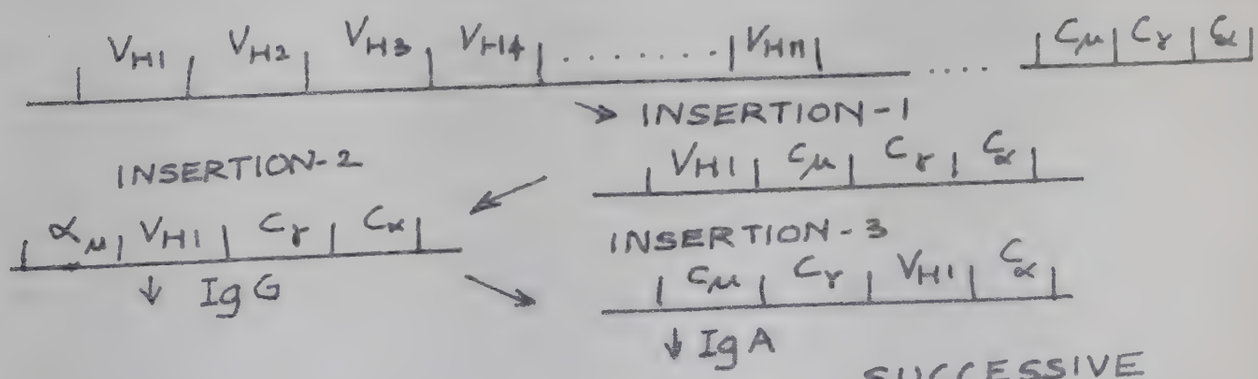


TRANSLOCATION OF (Ig) GENE

4.



SIMULTANEOUS INSERTIONAL MODEL



SUCCESSIVE INSERTIONAL MODEL

4(A & B)

V_H - C_H JOINING MECHANISM

These suggestions were based on the findings that sequences complementary to K light chain mRNA are found on two different DNA fragment.

Further, these sequences undergo covalent modification during differentiation of the antibody producing cell . When the DNA isolated from myeloma cells were analysed, sequences complementary to the K light chain mRNA are found on only one DNA fragment. (see figure I8)

The other experiment was based on the technique developed in 1973, one of the major advances in molecular genetics the recombinant DNA technique. The procedure was to insert a DNA fragment into a bacteria or even a bacteriophage and thereby, clone a single gene in large quantity. A V_λ gene was isolated from embryonic mouse DNA and replicated by splicing and using the above technique. This was done by S. Tonegawa and his group. The entire nucleotide sequence of the V_λ gene was found out. It was seen that C_λ and V_λ were not adjacent in the differentiated myeloma DNA. The untranslated DNA between the two regions is known as the intervening DNA. (see figure-19).

Philip Leder, Jonathan G. Seidman and Edward E. Max cloned both embroyonic and the active forms of Kappa, variable region and constant region genes as K gene constituted 95 %. of mouse light chain molecules (Hood et al 1967).

In 1971, Frankin and Frangione studied proteins of H-chain disease (HCD) and analysed C region of F_d fragment. They proposed on the basis of evidence that the C region may be controlled by separate genes. Amino acid analysis indicated, there are three large deleted sections in the HCD proteins. The deleted section also include almost all the C_H1 domain as well as part of the V region . This deletion led Frankin and Frangione suggest that several genes control the synthesis of H region. Moreover, a human alpha-1 chain appeared to have a deletion of the entire C_H3 domain (Despont et al, 1974). Interestingly enough human α1 chain had a deletion which comprised of C region and a portion of V region (Wolfenstein-todel et al, 1974). Does this mean that more than two genes are involved in the production of a single polypeptide? But the amino acid sequence analysis data of the H-chain

Figure : 18 : Gene shuffling is a phenomena which occurs in the course of differenciation and development of an embryo germline to an adult active gene. This was shown to occur by experiments performed with the help of recombinant DNA techniques. DNA is obtained initially from two sources a) a mouse embryo b) from a plasmacytoma (tumor) mouse. The DNA is then digested with the help of restriction enzymes. The fragments of DNA obtained with the above method are separated according to size by electrophoresis on an agarose gel and then transferred to a nitro cellulose paper.

Fragments that incorporate the constant-region gene (C) are spotted by incubating the nitrocellulose paper with a radio actively labelled probe. The probe binds to any germline or adult DNA having complementary DNA sequence. The hybrid structure is identified by autoradiography. It is found that the embryonic DNA gives rise to a single band which corresponds to a single 'C' gene. However, the adult DNA yields two bands, one band represents the germ line organization, but the other which is less bright and slow moving band in the gel corresponds to that of the other allele (see section), which is rearranged and carries the C sequence in the configuration of an active gene. As shown in the figure, the Gene shuffling occurs to eliminate one of the original cleavage sites and brings in a new site closer to C gene.

GENE SHUFFLING

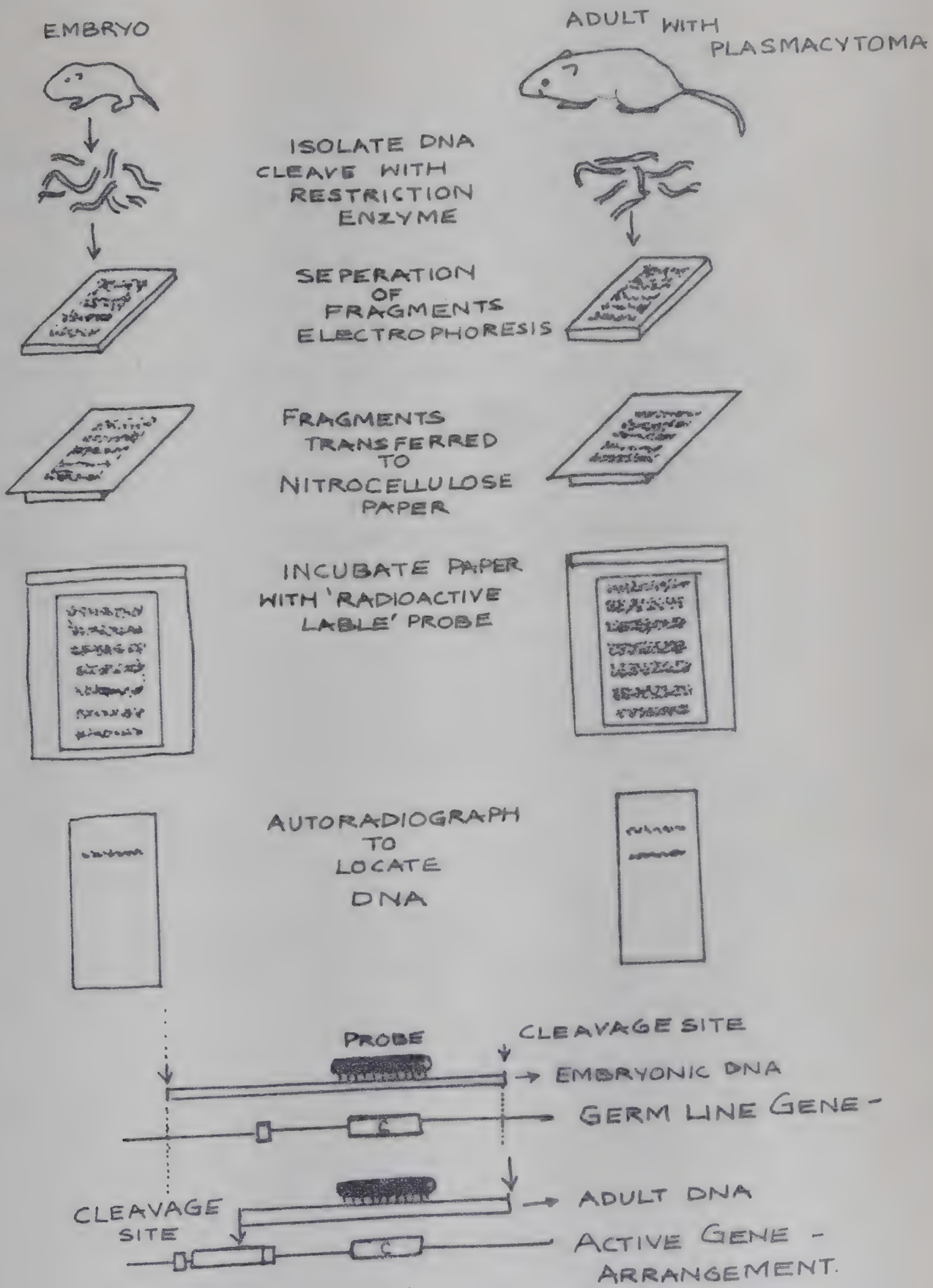


Figure - 18

IMMUNOGLOBULIN GENE CLONED

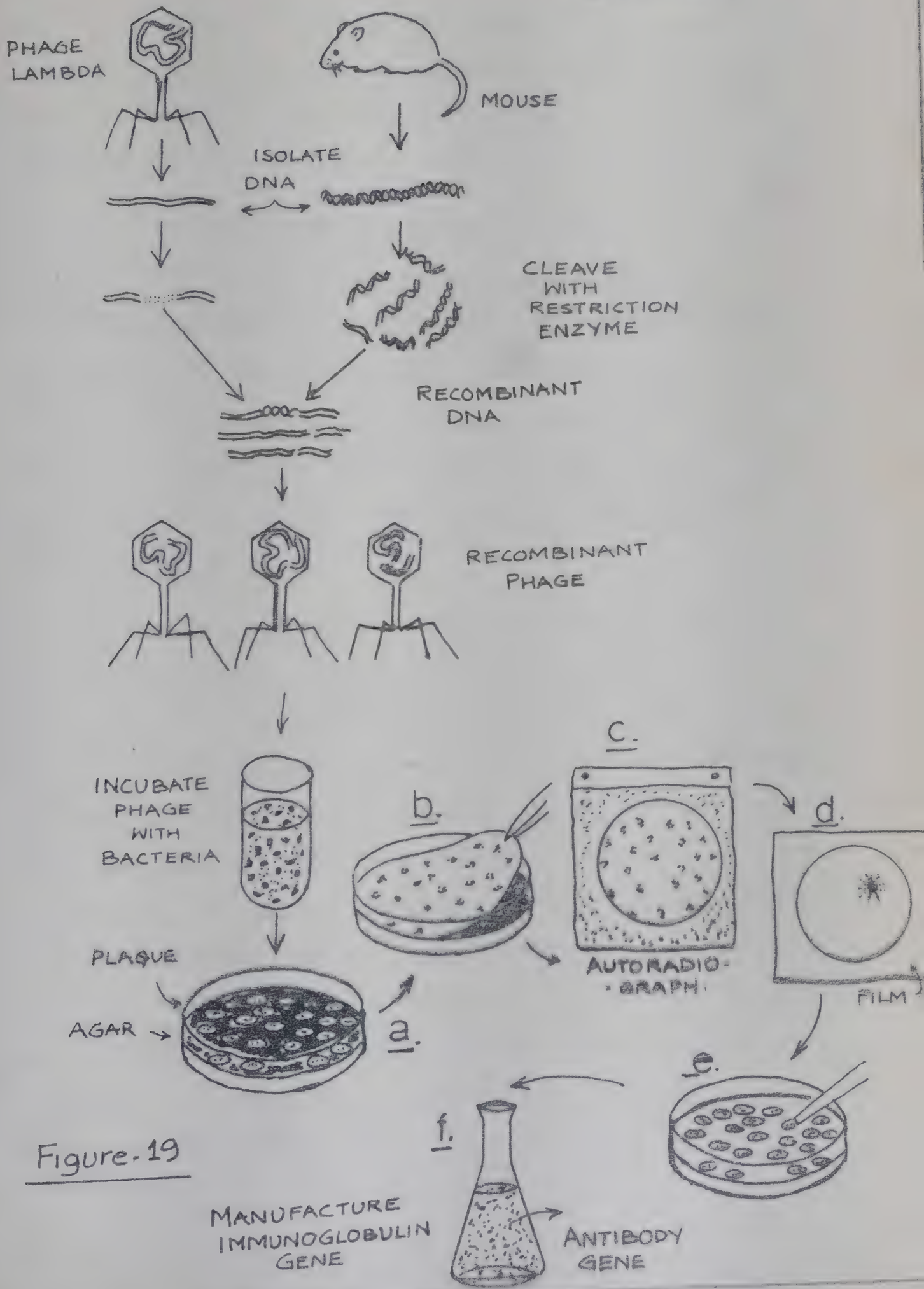


Figure-19

of the mycloma protein did not support this hypothesis. However, within a given subclass of human gamma chain, the C_H1 domains differ in amino acid sequence (Milstein,1970).

In analysing and comparing the variable region amino acid residues data, Wu and Kabat (1970)(as mentioned earlier) defined three htpervariable regions for the light chains in human, following which regions were even located in the H regions also. The interesting aspect of these hypervariable region was that, they occur at 'typical' positions called 'frame work positions'.

It was postulated that the genetic information for hypervariable regions is contained in the form of episomes in some extra chromosomal DNA (Wu and Kabat 1970). These hypervariable coding episomes are incorporated in the DNA of the structural genes in such a way that they are lodged in their respective 'frame work' positions. The process of incorporation involves translocation. This hypothesis has generated a lot of interest among immunogeneticists, and has been supported by observations made by (Wu,et al,1975 ; Capra, etal 1974 Kindt et al 1973 and Hopper et al 1976).

All these details regarding immunoglobulin genes clearly show, how different they are with respect to any biologically conventional gene . . . . .

An interesting point to be noted here is that, genes until 1977 were counted among the few fundamental biological problem that had been definitely solved. It was understood mainly due to the work on bacteria, that essence of life was contained in the genetic code, as mentioned earlier is a string of long nucleotide sequence (DNA).

But in 1977 it became possible to graduate from the genes of the bacteria to that of higher animals - and soon an unbelievable and complicated situation arose: the genes of higher animals carry the genetic code, not in a string of sequences but in small bits spread over a large chain of DNA molecule. What is more, it has since turned out that the interruptions between noncoding sequences (termed introns) are much longer than the coding sequences themselves (termed exons)! How can one account for these interruptions? How are the split genes got together to make 'sense'?

By a short margin, the first to discover these split genes of animal cells was Pierre Chambon in egg-white protein ovalbumin. At almost the same time, two other genes proved to have similarly complex structure. Philip Leder and Richard Flavell found out/that the gene for haemoglobin (of the red blood cells) was split into three genes each far apart! and Tonegawa, then at Basel Institute, showed the split gene nature of the immuno-globulin gene system.

In case of immunoglobulin gene system,it is well known that the gene organization would have to be much more complex because of the fact that there exists millions of immunoglobulin molecules qualitatively as against only a handfull, in case of proteins like haemoglobin etc.

Though the concept of split gene is recent, the unusualness of immunoglobulin molecules, as mentioned earlier, was felt much earlier. Various hypotheses, theories and experiments were proposed and inferred best suited to the then available data.

In the next few sections,a detailed description of, how the field of immunoglobulin gene structure, function, organization and expression developed and advanced, is made.

Coming back to our question which was raised - how are split genes got together? Applying with respect to immunoglobulin genes: How are V and C regions arranged and finally got together? To answer this question we go on to the next section.

4.2. The Translocation of variable and constant genes could be the mechanism for differenciation of an immunoglobulin producing cell.

Since the V and C regions are coded by separate genes in the germ line, their information must be joined at some stage so that the information put together makes sense enough to produce a immunoglobulin molecule. This linking of information biologically could be done at three different levels.

(a) Genome level (b) Transcriptional level (c) Translational level (d) Post translational level

The fourth possibility has been ruled out based on experimental evidences.
(Knopt et al 1967; Schraff 1967; and Milstein et al 1974

Though theoritically supported, no experimental evidence (Apte et al 1973) have been put forth to show its occurrance. It is now generally accepted

that at this level processing, does not occur.

The DNA-RNA hybridization technique - (Tonegawa et al,1976) strongly favours a DNA level fusion. Fusion at mRNA level could not be totally ruled out until this DNA-RNA hybrid technique was used. Now several models have been proposed to explain how V region and C region genes come together.

a)Dreyer and Grey (1968) postulated 'copy-splice' mechanism

b)Kabat (1972) postulated the looping out mechanism

c)Gally and Edelman (1970) proposed the translocation model

d)Smithies (1973) proposed the DNA net work scheme.

There are two interesting types of situation existing at the genetic level with respect to the variable and constant region genes details of which are given below.

4.2.1. Multiple Variable Genes and a single gene of constant type.

It was found out after analysis of various immunoglobulin molecules, that the C terminal of the

light chain is always identical within a given type of chain (Kappa or lambda) exception being of the allellic differences (see section 4.5),and that each light chain gives rise to a unique set of amino acid sequence in the N-terminal half (regions)which distinguishes each chain from all the others (Helschman,1975). The amazing degree of difference among V regions of K or chain have led to the acceptance that many variable chains must be synthesized by numerous germ line genes.

It was difficult to determine the exact number of V genes, until recently a highly handy and important tool - the recombinant DNA technology became available and was used in the study of immunoglobulin gene organization. The immunologist and molecular biologist used these techniques to characterize immunoglobulin gene segments in the genomic DNA. Southern blot type of experiments was one such experiment which played a very crucial role in the study of gene organization.

The total number of variable genes seems therefore to be determined by the number of subgroups multiplied by their average content of germ line V genes (number of bands obtained when the southern blot experiment (Brack et al 1978)we performed reveals)[Seidman et al (1978),Cory et al (1980a) Rabbitts et al (1980b).]

The total number of mouse V_K genes should be anywhere between 100 and 500 and that of variable - heavy type around 100. The important aspect here is the fact that not all these genes need be functional . There exists the pseudogenes:nonfunctional codons essential to describe the initial and termination sequences. (Joho et al 1982). (Huang et al 1981).

The lambda system is different. As it is well known that there exists four 'constant type' of chain, (Blomberg et al 1981), correspondingly ,there exists two $V_{\lambda 1}$ and $V_{\lambda 2}$ genes. They are believed to link up with either $C_{\lambda 1}$, $C_{\lambda 2}$ and $C_{\lambda 3}$. $C_{\lambda 4}$ appears to be a non functional gene (a pseudogene) (Blomberg and Tonegawa, 1982).

DNA hybridization studies has indicated that the constant genes are single genes in the germ line (Honjo and Kataoka 1978; Cory et al 1980b; Honjo et al 1981; Joho et al 1980; Blomberg et al 1981; Rabbitts et al 1980a).

4.2.2. Sharing of a single variable region by various constant regions.

Several facts suggest that a single variable region gene can be associated with many more constant

region gene. They are:

a) Single immunoglobulin producing cell may synthesize two or more classes of immunoglobulin molecule with same antigen binding site.

b) During an immune response, the primary reaction shows the predominance of IgM concentration in the serum which shifts to IgG during later stages and during secondary reaction, while their antigen binding site remains the same.

c) Majority of spleenic B lymphocyte express IgM and IgD simultaneously at the cell surface (Fa et al 1975) (Strober et al 1980) have shown this is not just a phase in B cell maturation when residual long-lived μ mRNA is coexpressed with new δ-mRNA.

The C_{μ} and C_{δ} genes are closely related and have lot of similarities.

d) The existance of the disease biclonal myeloma.

Various hypotheses have been proposed some of which were accepted at different stages, and later each one of them being superceeded as our understanding improved and experiments supported the recently proposed mechanism .

The problem of 'same variable region shared by different heavy constant region' as viewed by early immunobiologist are described below, but the recent proposal which has been more accepted will be described under (section 4.6).

a) The simultaneous translocation mechanism. This model proposes that copies of replicated variable gene may be linked to any given C_H gene. The differenciation then would be successive activation of complete V_H-C_H gene region by regulatory mechanism.

b) The successive translocation mechanism. This proposes that a given V_H gene may switch from one C_H region to another during differenciation. (figure-17.4)

c) The 'genetic switch' hypothesis was proposed by (Wang et al 1970a) on the basis of a study on monotypic IgM (Kappa) and IgG_2 (Kappa) from a single patient. According to this hypothesis, in the body during the differenciation of the immune system, a given clone of antibody producing cells can maintain its V region specificity while switching from the expression of one class of antibody to another.

Such a switch involved the repression of a C region gene (μ) and the simultaneous de-repression of another C region gene where as other structural genes V_L, V_H and C_L remain totally unchanged or unaltered. In this manner a single variable region both heavy and light can change the heavy constant region.

This proposal is supported by various experimental observations of

a) Pennetal,1970 and b)Nossal et al 1971 c)Bihrer et al 1974 d)Fair et al 1975 e)Pernis et al 1971 f)Kincade 1970.

A slightly different situation is found in mice (Vitetta et al 1975) New born mice have only IgM on the lymphocyte membrane. Beginning 10-15 days after birth,an IgD molecule like immunoglobulins start appearing on the membrane of the spleen lymphocyte and becomes the predominant cell surface Ig when the mouse is 3 months old. There was what then immunobiologist claimed a non antigen driven 'Genetic switch'. They explained the observations as a result of genetic switch.

So far the discussion was /centred on the non specific (i.e. organization irrespective of the various groups and subgroups of Ig chains) gene organization. The discussion on expression of variable-constant regions was essential for further analysis on individual Ig-gene family and sub family.

In the next section, the details of immunoglobulin gene family organization and expression are discussed. The structure of each gene family are analysed followed by various hypothesis proposed to explain the gene expression.

4.3. Immunoglobulin gene - Structure and expression

4.3.1.A. Organization of light chain genes.

a. The organization of lambda chain genes. Tonegawa and his colleagues (Bernard et al 1978; Tonegawa et al 1978)by using recombinant DNA technology have cloned and sequenced the V_λ genes.

Contrary to expectation, the nucleotide sequence of V_λ gene in embryonic DNA does not code for complete V region of the λ light chain but it stops at the codon number 97. The nucleotide sequence of V_λ gene in the functional form - (Plasmacytoma mouse) is the same as the inherited

form except that the nucleotide sequence terminates at a position corresponding to amino acid pumber 109, the end of variable region.

The nucleotides coding for amino acids 98-109 represent the J-segment gene. These experiments provide the support that cells can rearrange their genetic information which have been inherited.

These results also confirm the existance of V and C region genes occuring, in two different DNA fragment in case of embryo, and in one fragment, in case of plasmacytoma DNA. (see figure 21).

It has been shown that gene rearrangement occurs intra chromosomally and rarely, ever involves genes of other chromosome. This is a unique mechanism confined to immunoglobulin gene alone.

Nucleotide analysis show that its a regular feature with eukaryatic cell genes that λ chain genes contain intervening sequences. One of the two intervening sequences is made up of 91 DNA base coding for a leader sequence in the variable region and the other about 1,250 nucletides lying between J genes and C gene. (see figure 23.4) .

Figure 21

PROPOSED CHROMOSOMAL ORGANIZATION OF IMMUNOGLOBULIN GENE

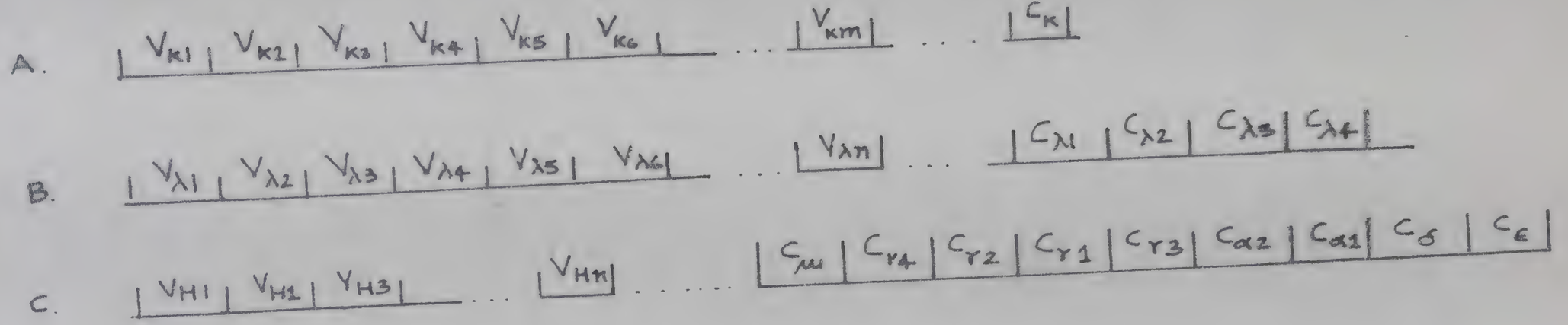


Figure - 21

b. The organization of Kappa chain genes: As mentioned earlier a large number of V region genes are identified in the embryonic DNA. In case of Kappa gene system the system includes limitless number of variable K genes, five J_K genes but just a single constant C_K gene (see figure 21). These J segment genes are separated from each other by about 300 nucleotides and form a cluster of 3000 nucleotide bases from C_K gene. Again supporting the V C separate gene hypothesis, no V gene is adjacent to any of the J gene. Two different groups (Sakano 1979b and Max et al 1979) have independently sequenced the region of DNA which contain J segment clusters (see figure 23.5). The exact number of V_K genes and their arrangement is not known, but analysis of V subgroup by (Leder et al 1978) has shown that there are up to six to eight different genes for each subgroup. Recent studies (as mentioned earlier)of

(Joho et al 1982) gives a possible estimate of the number V_K genes. (see section 4.2.1).

c. 'Variable-light-genes' organization: There are two important aspect of V_L-gene organization . 1) The immunoglobulin light chain synthesized is made to carry a 'leader sequence at the N-terminal (Burstein et al 1977, 1978) . The analysis of the leader sequence

show that it is made up of 20 amino acid which is cleaved off during the maturation process of the polypeptide. 2) As mentioned earlier, the first ever V_L genes were isolated from embryonic DNA. It therefore became necessary to clone and analyse true germ line genes, because it was clear that V_L genes were not completely assembled in embryonic DNA. Using the southern blot technique it was shown (Early et al 1980a and Gershenfeld et al 1981) that germ line immunoglobulin genes are not composed of separate segments but are build as embryonic ones. (see figure 20).

d. 'Constant-light region' gene organization: As mentioned earlier cloning and sequence analysis of C_K and C genes revealed the J regions coding for the characteristic position (Black et al 1978). The $J\lambda$ gene segment is of 39 nucleotides and is positioned very next to $V\lambda$ gene and $J\lambda$ and $V\lambda$ form the complete V(λ) segment gene in the lambda producing plasma cell.

The C_K gene are quite similar to that of C

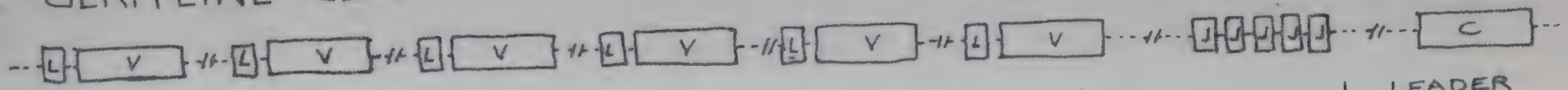
There exists 5 C_K J segment genes. The fifth J_K segment gene has an 'atypical configuration' (Max et al, 1979, Sakano et al 1979 .Lenhard-Schuller

Figure : 20 : The active gene for LC (light chain) is organized by a process of somatic recombination and RNA splicing (example here is a Kappa chain). The basic components of active genes are present in the germ-line, but in a disorganized state, the germ line (1) is variable region of the chain (encoded by V and J sequences) the constant region by a C gene .Each of the V gene has a leader sequence prefixed to it. The L/V segments are followed by 5 J segments, many intervening sequence and then by C region genes.
During lymphocyte cell development a L/V sequence is recombined with one J sequence along with the C gene to produce an active gene (2). The entire gene is transcribed into a hnRNA(3) the (IV) sequences and extra J segments are spliced out(4). This is then translated into a protein; the L.C. precursor(5) Leader is cleaved away as mature chain(6). [adapted from Brack et al 1978].

Figure : 20.1 : Active gene for (LC) example lambda.

LIGHT CHAIN - GENE

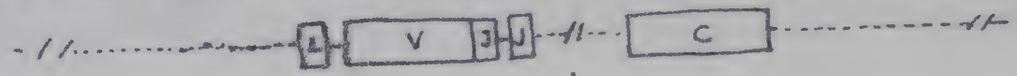
1. GERM LINE - GENE



L- LEADER
J- JOINING
V- VARIABLE
C- CONSTANT
} REGION GENE

V/J RECOMBINATION

2. DNA-LEVEL (AFTER RECOMBINATION)

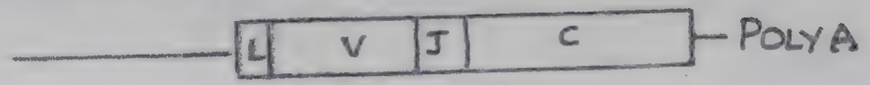


TRANSCRIPTION

3. RNA-LEVEL (AFTER TRANSCRIPTION)

SPLICING

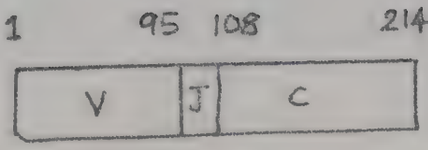
4. RNA-LEVEL (AFTER SPLICING)



TRANSLATION

5. PROTEIN LEVEL (AFTER TRANSLATION)

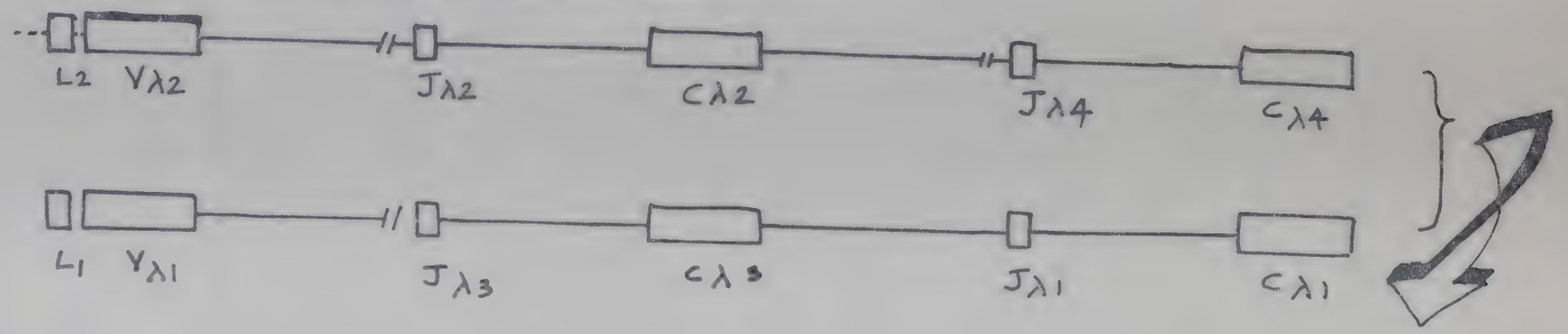
6. PROTEIN LEVEL (REMOVAL OF LEADER)



LIGHT CHAIN GENES : λ system

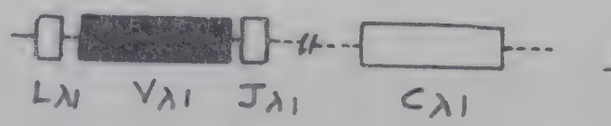
GERM LINE CONFIGURATION :

1.



V/J RECOMBINATION :

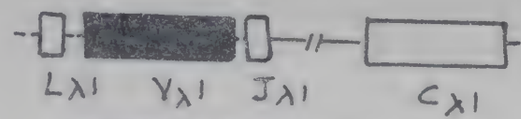
2.



} DNA LEVEL

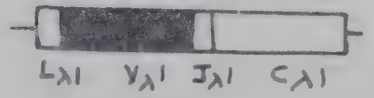
TRANSCRIPTION :

3.



SPLICING :

4.



} RNA LEVEL

TRANSLATION :

5.

REMOVAL OF LEADER :

6.

} PROTEIN LEVEL

FIGURE : 20.1

4.3.1B. Expression of light (Kappa and lambda)genes.

Cloning techniques performed by (Rabbitts 1978; Brack et al(1978); Bernard et al(1978) Schible et al 1978) have thrown some light on the expression of lambda genes. A complete functional lambda gene contains two non translated intervening sequences:

a) a short sequence of 93 base pairs separating the coding main variable gene from the hydrophobic leader sequence.

b) a much larger sequence seperating V_λ from C_λ

The expression of Kappa genes follow a similar basis. But the one basic difference which exists is that in the five J segment genes, the expression being a combinational association of the V_K gene to any four other J segment thus leading to a amplification of the stored variable gene information of the germ line. (see figure 20).

4.3.1.C. Intra codonal V-J joining

The variable Kappa genes . end at a position corresponding to aminoacid 95. (Germline or embryonic DNA) . The beginning of J-Kappa is with residue no 96.

It is expected that V_K and J_K be joined at this junction. The exact joining need not be the same (Max et al 1979).

There exists considerable variation at the joining point, but the essential aspect of the V-J linkage is that the joining takes place to form a functional V Kappa region gene and this result's in a correct reading frame. Out of phase reading results in premature termination of V region. The false reading and slippage of V-J joining seem to occur to the right of 95th codon residue because of the amino acid residue at that position which is proline. (Weigert et al 1978). It seems apparent that this amino acid is very essential for structural integrity of the Kappa chain (Rudikoff et al 1980; Weigert et al 1980) (see figure 23.4 and 5).

4.3.2. Organization and expression of Heavy chain genes

It is expected that the gene arrangement of heavy chains are quite similar to that of the light chain genes. There exists several V_H, C_H, J_H genes multiple genes (Roa et al 1979). Although some sort of similarity does exist, there exists one characteristic difference in the positioning of the V_H gene. A short intervening sequence, about 80 bases, separates the coding sequence of hydrophobic leader and the

major variable region. The mechanism for V-J linkage is likely to be the same as the light chain organization, but the number of V_H and J_H genes were not exactly known until recently. (see figure 21). The constant (heavy) gene region is fairly complex containing eight different C_H genes coding for constant region parts of various heavy chain. Very recent analysis (Newell et al 1980; Sakano et al 1980 and; Gough et al 1981) demonstrate the presence of four J_H genes as a possibility.

As in the case of Kappa and Lambda J segment genes each of the four J_H gene could code for a specific frame work V_H region. A DNA segment coding for the hypervariable region-3 was not present with any of the four J_H gene. Early and Hood 1980 reasoned that this missing DNA segment must be supplied by a stretch of embryonic DNA and they called it D(for diversity) gene.

Further analysis of functionally rearranged V_H gene showed that the V_H gene segment joined to the D-DNA segment and were followed by one of the four J_H gene (Bernard and Gough 1980). The correct or actual location as well as the number of D-gene is not exactly known as yet. (Kurasawa et al 1981;

Kurosawa and Tonegawa 1982) (see figure 22).

Thus activation of a V_H region gene requires at least two joining events

a)a V_H gene segment to be joined with a D gene (V-D) linkage

b)The linking up of a D gene to any one of the four J_H gene (D-J) linkage

The combinatorial assembly of a complete V_H region inclusive of a V_H gene segment, a D segment and a J_H segment amplifies the germ line information.

The intra codonal V-J joining as described for the light chain system is also similar in operation during V_H-D and D-J_H linkages (Rao et al., 1979; Schilling et al 1980).

4.3.3. Mechanism of V_L-J_L and V_H-D-J_H linkage

Several mechanism have to be proposed to account for V_L-J_L and V_H-D-J_H linkage. The location and orientation of V genes corresponding to their C region gene is not known. But it has been assumed that the orientation of V gene cluster is the same as that of C gene. The C_H and V_H genes are present on

HEAVY CHAIN-GENE

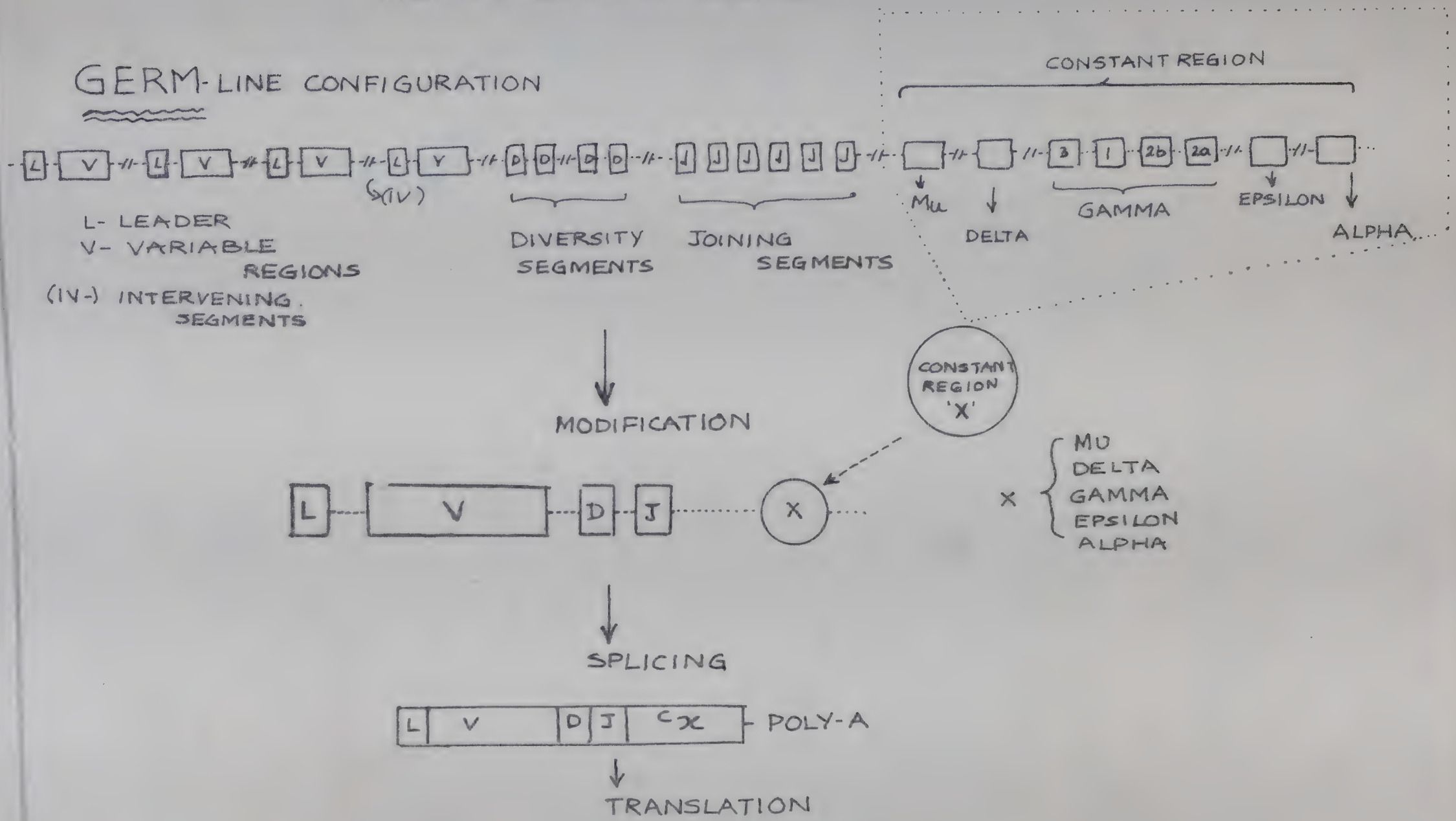
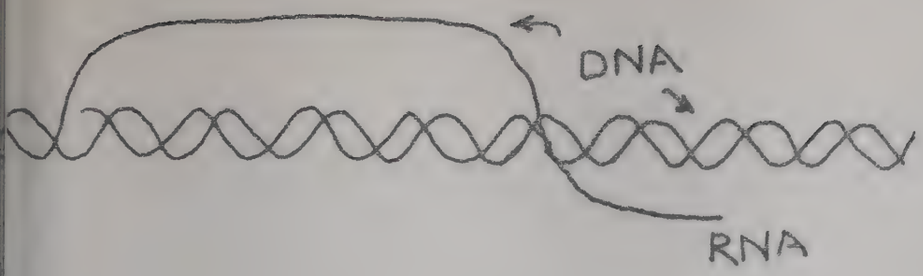
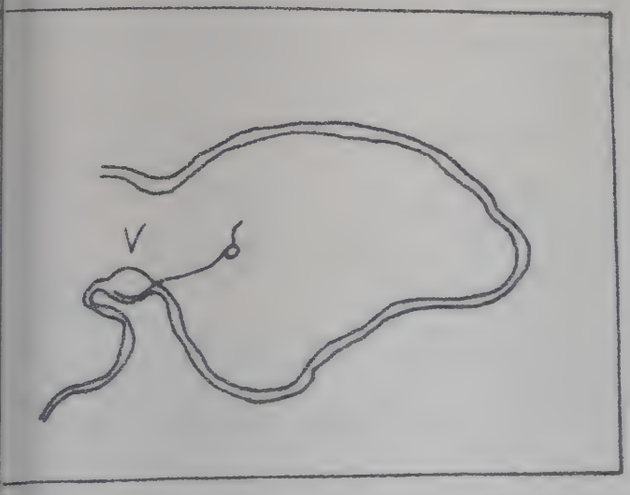


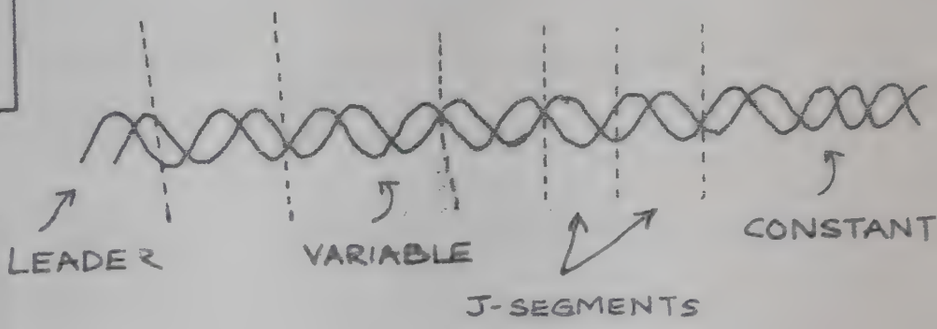
Figure - 22

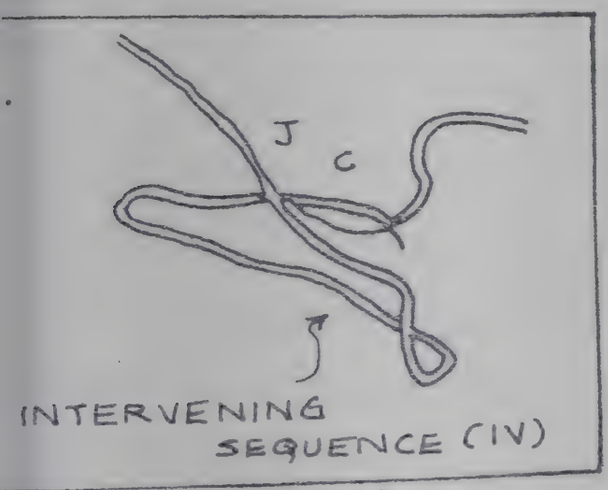


IMMUNOGLOBULIN GENE

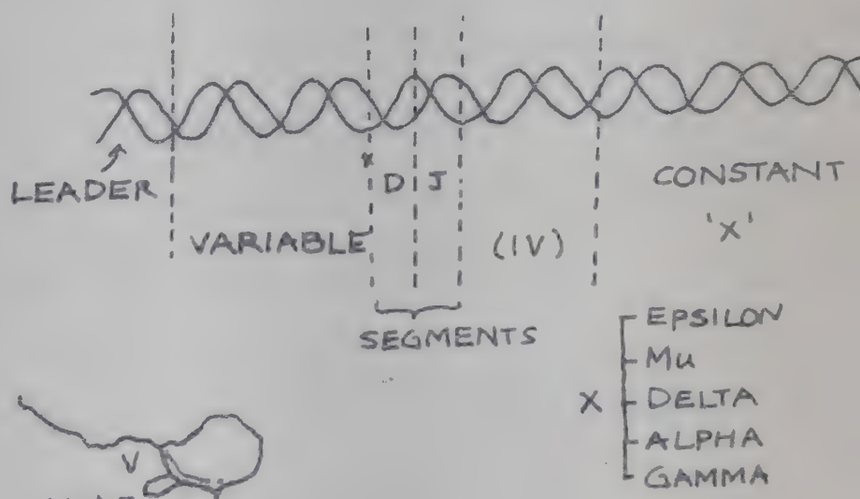


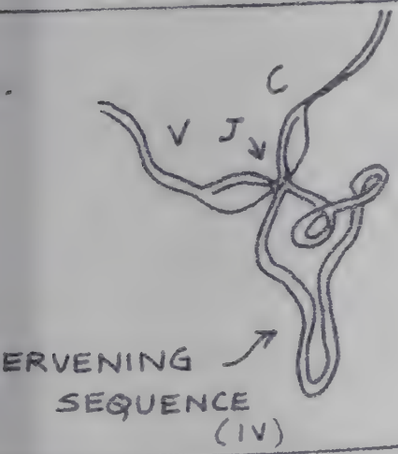
* LIGHT CHAIN - GENE *

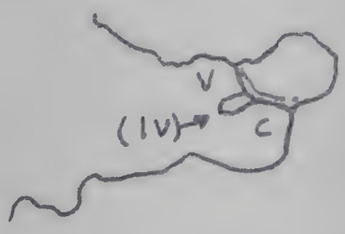




* HEAVY CHAIN - GENE *







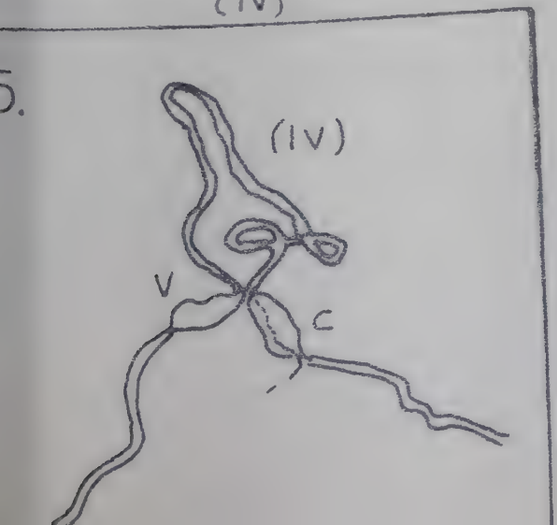


Figure - 23

different chromosomes therefore during the process of V_L-J_L and V_H-D-J_H linking up, the DNA between V and J segment gene are deleted (Seidman et al 1980 ; Sakano et al 1981) and if D segment is situated between V_H cluster and the J_H-C_H gene cluser, V_H-D-J_H link up would probably occur as a two step mechanism with deletion of corresponding segments of intervening DNA (Van Ness et al 1982).

As mentioned earlier, certain features of the sequences of the light chain genes have been conserved and seem likely to be of functional significance (in specific, a pattern of signal termed 3 prime or downstream side of J gene)(Early et al 1980a; Sakano et al 1980). Each sequence has a length of nine nucleotide (Nanomer) of which a large proportion are either bases Adenine or Thymine. This anomer is followed, at an interval of either about 11 or 2x11 bases, by a seven base pairs sequence or heptamer CACTGTG or GTGACAC. These two sequences the anomer and the heptamer can be pictured as forming a 'stem' like structure in which sequences would be complementary according to base-pairing laws bringing V and J genes together at the base of the stem. A mechanism of unequal sister chromatid exchange has

also been proposed and could account for gene translocation (see figure 24.A).

The flexibility of the recombinational system, although strong and powerful enough to create diversity, but does have its price. The V and J genes are/ brought together rather aberrantly yeilding an inactive gene. This once again may in part explain allelic exclusion.

It is also possible that the lambda light chain genes serve as a fail-safe system for misjoined Kappa genes.

Findings of Early et al 1982; Kurusawa et al 1981; and Sakano et al 1981 support the notion that the structure: heptamer-spacer-decamer following each V and D gene, precede's in an inverted fashion and each D and J gene segment is very essential as they hold the key in the immunoglobulin gene rearrangement (see figure 24.B).

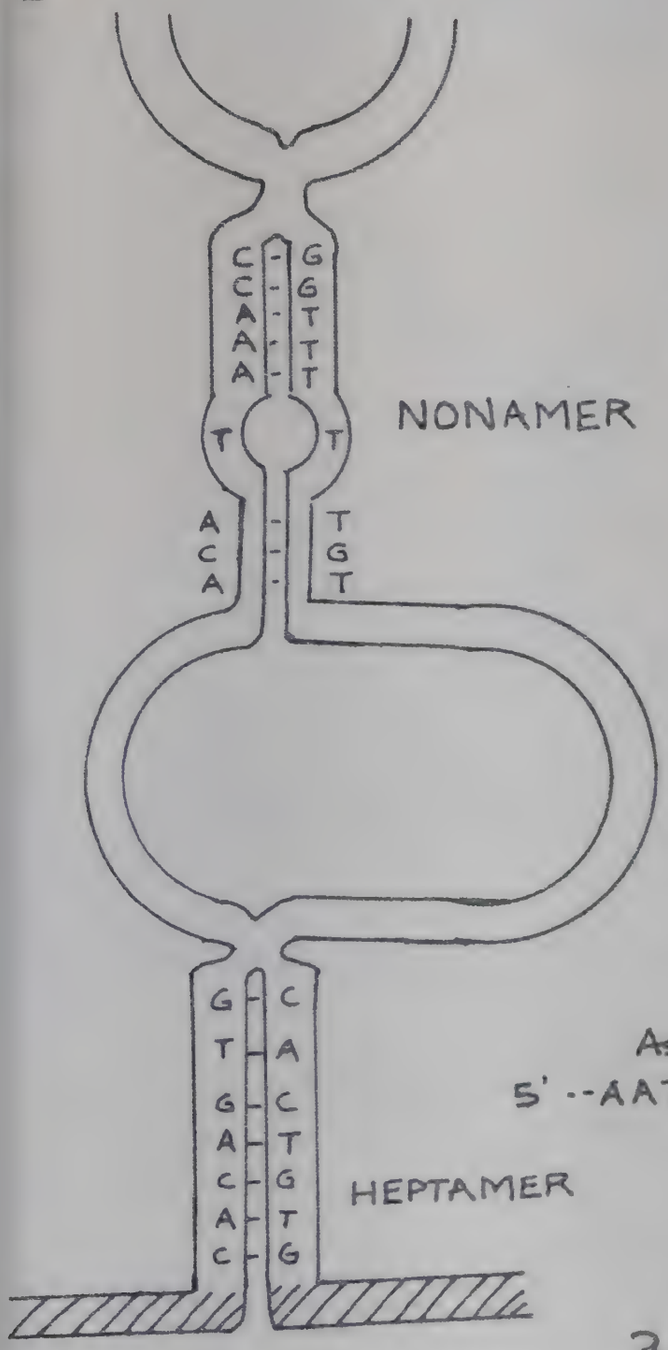
Earlier studies using allotypic markers suggested that the V-J and V-D-J joining are intra-chromosomal [(Dubinski S.J. 1969, Kindt. T.J. et al (1970) and Landucci Tosi.S et al (1970)]. An intra-chromosomal recombination is said to occur according to any one of a combination of several different basic model

Figure - 24-A

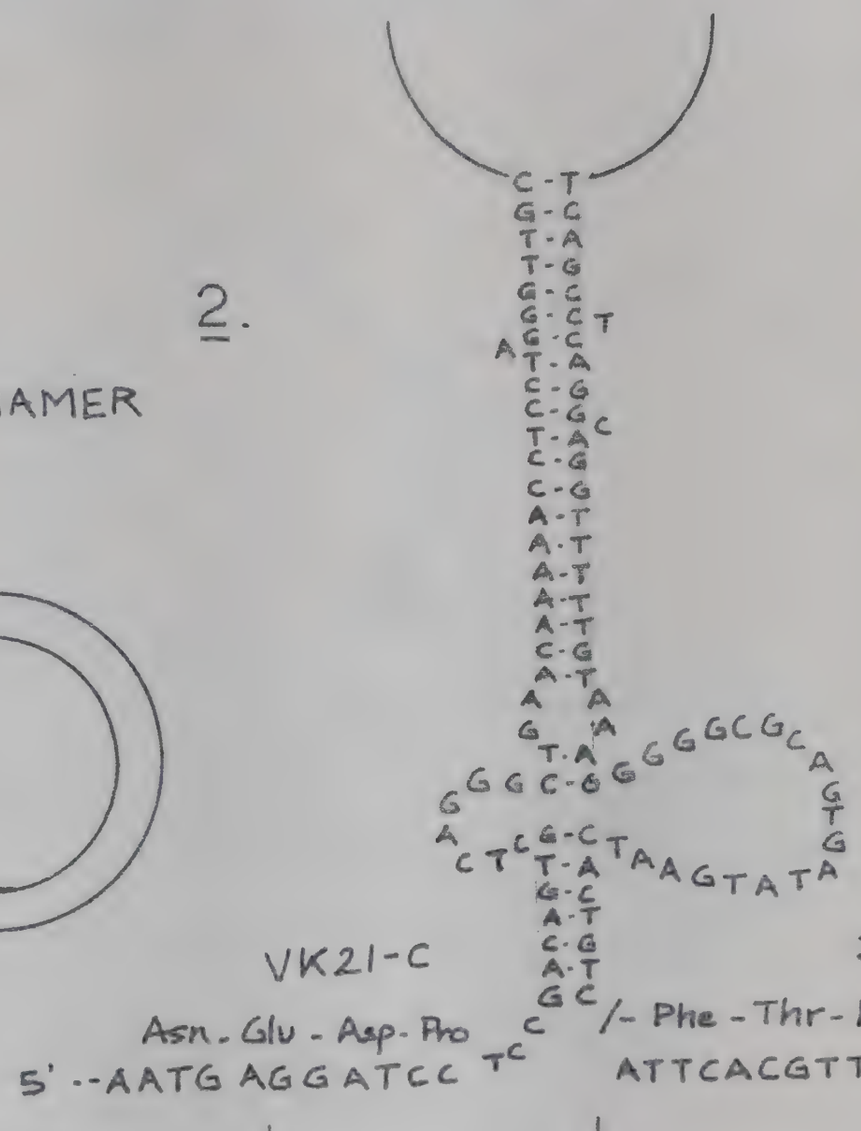
RECOMBINATION SIGNALS

(LIGHT CHAIN)

1.



2.



3.

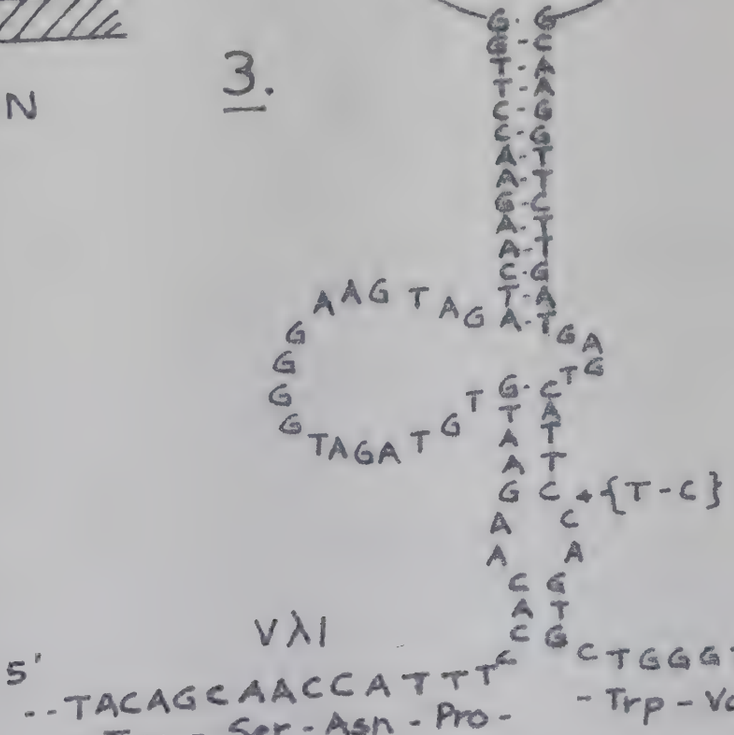
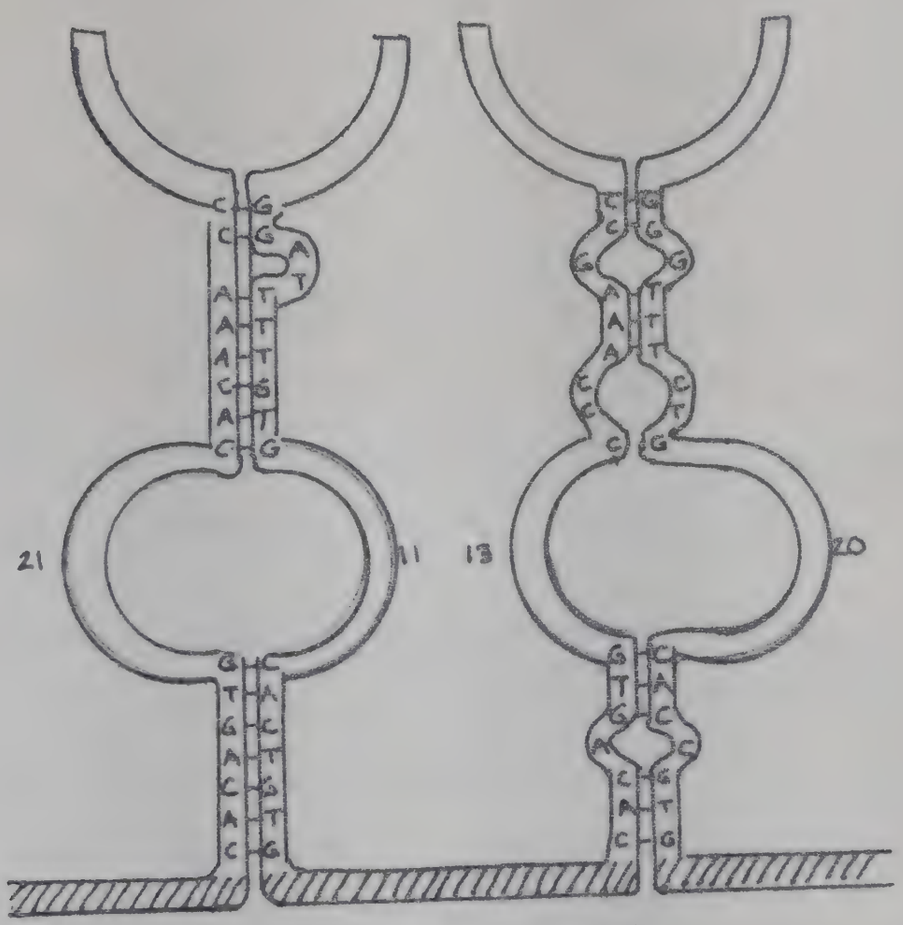


Figure - 24B

RECOMBINATION

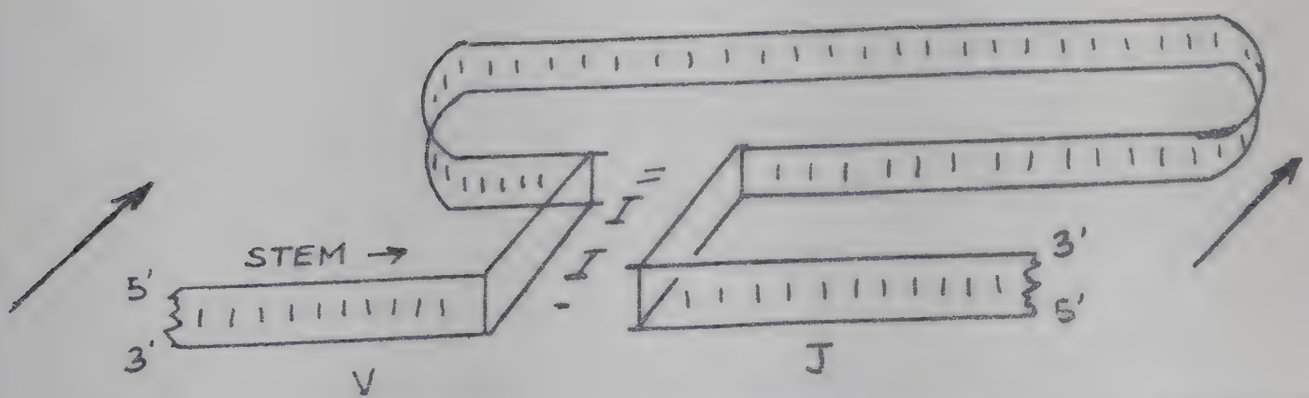
(HEAVY CHAIN) SIGNALS

1.



HEAVY CHAIN

2.



"HYPOTHETICAL" 3-D STRUCTURE OF THE STEM STRUCTURE

(Tonegawa S et al (1977)). The first southern experiment however favoured the out deletion model :as [mentioned in the last paragraph Sakano H et al 1979] and was confirmed by Seidman J.G., et al (1980).

However Zachau et al (1981, 1982) have shown that looping out deletion need not be the only mechanism. They found that V.J. linkage followed an inversion pattern : a tail to tail configuration. This was confirmed by Van Ness et al (1982).

However Lewis et al 1982 showed that a combination of deletion and inversion could explain all the existing findings. This was supported by the recent finding of a truly, inversion product Kappa myeloma which favours the involvement of inversion also. [Hochtl J and Zachau H.G. 1983].

All the above mentioned findings are relevant in considering the V-J and V-D-J joining mechanism at fine structure level. Based on these findings Alt and Baltimore (1982) have proposed a model. Details of this model are beyond the scope of this review.

Thus to conclude, the actual mechanism of V-J or V-D-J joining is not known as yet. But all those which have been described under this section as various 'models' could be the main possibilities.

So far the structural and expression aspect of immunoglobulin genes were analysed, the discussion included the Gene rearrangement and individual chain subgroup genes and their characteristic structural features. In brief, here is a summary of the variable-constant region genes organization.

The light and heavy chain are made up of two types of regions: variable and constant regions. The combination of these two results in the variety of immunoglobulin molecule. The variable region genes in both the cases are inherited as mini genes in a discontinuous fashion. Two such genesegments (V_L and J_L) form a complete light chain variable region gene. The heavy chain variable region gene is formed by three independently inherited gene sequences (V_H,D and J_H). The combinatorial assembly of a particular V_L to that of another J_L in case of light chains and similarly any specific V_H to that of another D and J of heavy chains and in turn a complete light chain to that of a complete heavy chain region gene explains the great depth of rearrangement possible during antigen independent development (see figure 23).

The challange that, the organization and expression of the immunoglobulin genes has posed to us is that of

the understanding of the diversity of the immunoglobulins synthesized by organisms under different 'immune stress' conditions. Several mechanisms are utilized to amplify and modify the genetic information, related to immunoglobulins, to increase their versatility and thus produce an enormous diversity amongst themselves.

Theoritically pxq types of immunoglobulin molecules are possible for a given 'p' light chains and 'q' heavy chains, irrespective of the factor which of the two heavy or light) chains would be functionally active in the combined state. (antigen-antibody reaction or effector mechanism or complement binding).

In order to picture the possible mechanism responsible for the immunoglobulin diversity, each of the light and heavy chain, variable and constant region genes are considered to be inherited as 'minigenes' [originally proposed by Kabat(1978)]. Two such gene segments (V_L and J_L) form a complete light chain variable region gene. Three independently inherited segments (V_H,D and J_H) account for the heavy chain variable region gene. Combinatorial assembly of a particular V_H and V_L with a given J_H or J_L and a D with any one of the 8 C genes respectively , leads to a very great amplification of not only the variable

genes but also the immunoglobulin molecules as a whole.

As mentioned under section (4.2.1) an approximate estimate of the number of various genes have been proposed. Considering these estimates, if 100 V_H gene segments combine with four J_H gene segments and perhaps any one of the ten different D gene segments (the actual number of D genes is not known), a total number of 4000 different V_H genes can be generated. Any 'slippage' occuring during the process of V_H - D and D - J_H linking introduces 'differences' at both junctions. Allowing around three 'difference' situation (which could be an under estimation) to exist 40,000 V_H genes could be generated! There exists a lot of doubt since only a few evidences to support, regarding the possible occurrence of the mechanism of any light chain combining with any heavy chain to form an immunoglobulin molecule. If this were true, then 1×10^8 antibody or immunoglobulin molecules can be generated! For details regarding gene movement and expression see Honjo T et al (1983).

Apart from the above mentioned phenomena of diversity, there exists another event which is also a consequence of the immunoglobulin gene organization and rearrangement. During the antigen-dependent phase of lymphocyte maturation, immunoglobulin molecules of

different C regions (effector function) but with the same antigen binding specificity are produced in progenies of the same B-cell. This event of or the process of heavy chain switch is mediated by another form of immunoglobulin gene rearrangement. In order to understand the 'genetic switch', a brief discussion on an interesting topic:'the genetic analysis of immunoglobulin molecule using serum markers 'has been done. The serum markers are used to detect 'typical' positions in an immunoglobulin molecule and thus help us to differenciate between switched immunoglobulins .

4.4. Mammalian immunoglobulins are coded by three unlinked clusters of genes

Genetic analysis of immunoglobulins using serum markers show that there exist three unlinked clusters of genes. They are:

a) Allotypes: Allotypes are immunoglobulin variants which are inherited as alternatives (i.e. alleles), by mendelian pattern of segregation. The various allellic forms were first observed as pairs Todd (1963) present in IgG and IgM and were designated a1 and a3, but subsequently were also found in IgA molecules. Feinstein (1963), Serological and biochemical analysis demonstrated

that these variants were associated with variable region of the heavy chains.

The sharing of V region alleles by the Heavy chains gamma, Mμ and alpha supported the concept of V-C two genes one polypeptide. Later, after amino acid sequence analysis (Mole et al 1975) of H chain it was found that there were large scale amino acid substitution, never been observed so far in any other protein system except in C_K - perhaps (Appella et al 1969; Nezlin et al 1974). It was postulated that the a-group allotype of rabbits may actually be allellic forms of a hypothetical 'regulatory gene' and each of these alleles regulated the expression of a set of closely linked V_H gene (Wang 1975). It was predicted that C_K region would have similar genetic setup.

Three human C_K region variants were termed 'Inv' markers: serological identification of these three Inv markers in family and population studies has shown that these polymorphisms behave like three allele's of a single gene. The Inv markers have been changed to km and correlate with simple amino acid substitution at positions given below:

Km allotypes	amino acid position	
1.2	153	191
3	Ala	Leu
1	Ala	Val
	Val	Leu

(data from Milstein et al (1974)

Thus in short allotypes are variants that exhibit a mendelian pattern of segregation and are localized to C regions and are consequently mapped on C genes . They have been found on and K chains and also heavy chain classes and sub classes (see table VI) (for complete details R.Mage et al 1973). (See table VI).

Table - 6

Allotypes of human

Immunoglobulin family	Alleles
Kappa	Inv(1) Inv(2) Inv(3)
Lambda	none
Heavy	
G_1	Gml, Gm(non-1),Gm3,Gm 17: Gm2,18,20

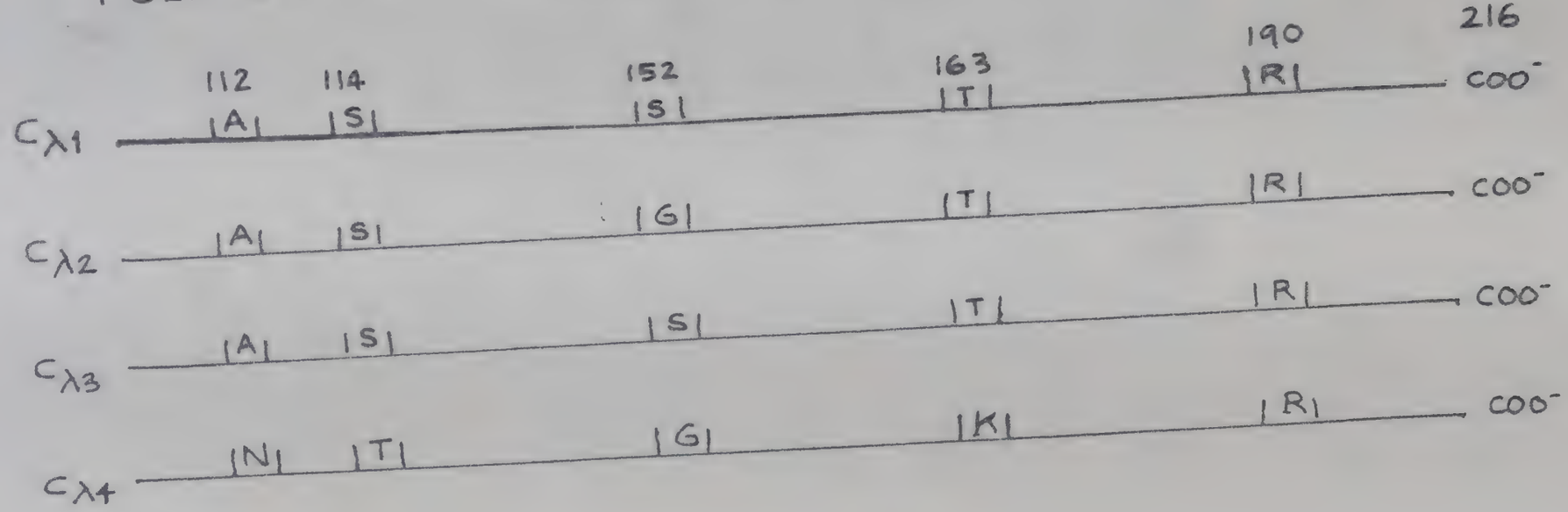
Table - 6 (contd.)

Immunoglobulin family	Alleles
G_2	Gm23, Gm(non23)
G_3	Gm21 Gm(non21) Gm11 Gm(non11)etc.
G_4	Gm(4a) Gm(4b)
A_1	not identified
A_2	Am(1) Am(-1)
D	not identified
M	-do-
E	-do-

b. Isotypes: Isotypes are determinants that characterize each class and subclass of heavy chain and each type and sub type of light chain. Isotypic antibodies are produced by immunizing any mammal with the antibodies of the same species, and is useful in detecting basic differences between isotypes, since members of the same species will show or express the same isotype. In case of humans there exist C region variants (see figure 25). Isotypes can reflect limited variation of a few residues as in C regions which presumably arose by recent gene duplication. Isotypes play as a handy tool in our understanding of Ig diversity.

Figure 25 - ~~26~~ 25

POLYMORPHISM REPRESENTING - ISOTYPES - CODED BY 4 Cλ GENE



c. Idiotypes: Idiotypes broadly can be difined as the determinants that distinguish one V domain from all other V domains. This is done by raising homogous antibodies between the same species or very closely related one. Idiotypes are found to be of great importance for mapping V region genes.

Idiotypes was originally defined as a unique antigenic determinant found on the variable regions of the immunoglobulin molecules produced by a single clone. (Oudin 1966). Since this definition could be misleading compared to our present understanding with respect to structure function relationships in immunoglobulin molecule, our present definition of idiotype is that, it is the antigenic determinant formed by the structural combination of V_H and V_L regions. This definition of an idiotype is also somewhat unclear because, the antigenicity of amolecule depends upon how it is used to induce an immune response, so that different antigens may detect different idiotypes on the same molecule. Idiotopes which are typical of any given individual are known as Idls, and those that are inherited are known IdXs or cross reacting idiotypes.

Idiotypes in Practice: There are two types of idiotypes which have been of extensive use . They are known as anti-Idiotypic reagents (anti-Idls and anti IdXs).

In case of anti Idls. They can be used to confirm the V region homology and to estimate the largeness of the group in the immunoglobulin molecule. In case of anti Idxs, they are of much more use compared to that of Idls and are used at gene level for identification(see Table VII) (for details M.Potter 1977).

Table - 7

Commonly used cross reacting idiotypes of mice

Idiotype	Locus symbol	Reference antibody	Reference strain
A_5A	Igh-Sal	Antibodies to CHO on streptococci	A/J
Tl_5	Igh-Pc	Antibodies to phosphoryl choline	BALB/C
$J_{55}8$	Igh-Dex	Proteins $J_{55}8$ and MOPC 104E; anti-dex trim antibodies	BALB/C
ARS	Igh-Ars	Antibodies to P-azo phenyl arsonate	A/J
NP	Igh-NP	Heterocyclic antibodies to NP	$C_{57}Bl$

Adapted from Green M.C. (1979) Immunogenetics 8; 89.

Genetic analysis of allotypes, isotypes and idiotypes have led to several conclusion about the antibody gene organization.(at subchromosomal and chromosomal level).

Family analysis of Ig genetic markers in man as well as other animals show that Immunoglobulin polypeptides are coded by three unlinked clusters of autosomal genes. One cluster codes for heavy chains of all classes; the second and the third code for Kappa and lambda chains respectively. These gene clusters are called the Heavy Kappa and Lambda gene families respectively. Each linkage group consists of a family of V region genes and one or a family of C region genes. In man the Kappa group have just a single C gene, the lambda group at least four C region gene. (Hess et al 1971, Fett and Deutsch 1975). The H group has at least nine C region genes. (Gally and Edelman 1972). The number of V region gene is still a matter of controversy.

But most of the workers agree that it should be much larger than the number of V region subgroups within each linkage group (Cohn et al 1974) (see section 4.2.1) and 4.3.1A). The evidence for this linkage grouping is given by (Pink et al 1971) in man. (Kelus and Pernis 1971; Mage et al 1971) and in rabbit (Herzenberg et al 1968) in mouse . From this it appears

that all H groups are linked, while in man (Stein berg et al 1964) and in rabit (Kindt 1975) the C region of Kappa lambda and H chain are unlinked.

Genetic analysis of idiotypes in mice has demonstrated twelve V_H markers that segregate in the mendelian fashion and are closely linked with the C_H allotypic markers. Experimental results also indicate that multiple V_H genes are linked to the C_H gene cluster in the DNA of mice. (Pawlak 1973; Eichman 1975).

It has been demonstrated that the H chain V region allotypes are linked to lambda chain C region allotype in rabbit also.

This linkage relationship implies that only V and C region genes located in the same chromosome can be fused together to code for a single immunoglobulin polypeptide chain.

Allotypes can be classified into two categories, designated as simple and complex allotypes. The simple allotype as mentioned earlier differing only with respect to few amino acid residue at one or a few position . But with respect to the complex allotype the amino acid residue vary in a multiple fashion.

This topic on allotype has been raised again because of genetic or evolutionary mechanism underlying their origin. The genetic mechanism involved has helped us in our understanding of the antibody genes. Complex allotypes are difficult to explain and three different models have been proposed to explain their origin. For detail description and analysis of complex allotype please see (G.Gutman et al /(1975). Thus it can be seen that serum markers, form one of the most important, of the various techniques used by immunologist.

The next topic of analysis is that of those, individual immunoglobulin producing cells, (from animals heterozygus for immunoglobulin allotype), to make only one of the two allelic forms potentially available - the allelic exclusion .

4.5. Allelic exclusion - specific gene activation and gene expression

The antibody or immunoglobulin genes exhibit an important regulatory phenomena known as allelic exclusion, which superficially appears similarto the X-chromosome inactivation (Lyon 1968) in which it was found that all X-chromosomes inherited, except one were inactivated during early stages of development. The principle of

allelic exclusion seems quite alright in the 'macroscopic' state chromosomes level, but this mechanism at the molecular level is so complex that not much of it is known or understood as yet.

Genetic and biochemical studies in higher organisms demonstrate that in a given individual (heterozygous for genetic markers) a given lymphocyte clone expresses only one light chain and one heavy chain allele which can be said as 'typical' of that clone. This behaviour is in contrast to all the other mammalian conventional protein studied such as allelic forms of haemoglobin (of which quite a lot has been understood), which are produced co-dominantly in the cells of the individual.

As mentioned earlier structural and genetic studies have indivated that multiple genes are involved in the synthesis of V and C regions of Ig polypeptide chains (Gally and Edelman 1972, Wang 1974). Atleast nine C region genes γ_1, γ_2, γ_3, γ_4, α_1, α_2, μ, δ and ε) for human H hchain have been described. Although the exact number of human V region genes has not yet been established, it is well accepted that it is likely to be larger than the number of subgroups described (Fundenberg et al 1972 and Cohn et al 1974) also see section (4.2.1.) . The genome of each lymphocyte producing antibodies would thus

have multiple genes coding for each V_H, C_H, V_L, C_L regions, and yet as its is known under normal conditions, any given clone of lymphocyte synthesize only one specific kind of immunoglobulin molecule. This phenomena was termed specific gene activation (Steinberg 1970).

The detailed analysis of published amino acid sequence data show that there exist sequence homology within a 'chain family' at inter species level, as compared to another chain family of the same species elaborating this point - V region of family in case of human has much large sequence homology when compared with V region of family in chicken or turkey species against V region of Kappa family in man) itself. (Wang 1975). This observation led to the idea that all vertebrates share a large number of similar if not identical V region genes and they seem to have been evolved from a ancestral or a primordial gene. Different sets of these genes are assumed to be expressed in different species. Regulatory genes decide the genes those are to be expressed - the concept of 'differential gene expression'. (Wang 1975). There exists various evidences which indicate the possibility of the existance of 'regulatory genes' controlling the expression of Ig structural genes.

4.6. Allelic exclusion and immunoglobulin gene rearrangement

As mentioned earlier the mammalian cells at a genetic level express both alleles, but there are only two exceptions to this general rule (Pernis 1965).

Comparative southern blot analysis between embryonic and myeloma DNA showed that two C Kappa bearing bands in case of myeloma DNA, and one of which was identical to the one found in the embryonic DNA, this second new band showing the rearranged and expressed V Kappa and C Kappa genes. In case of other myelomas constant Kappa arrangement at germ line level could not be found but only the rearranged form could be (Hozwmi et al 1976 ; Rabbitts et al 1978; Sternmetz et al 1980). This only could mean that there could possibly have been a loss of homologus C Kappa gene bearing chromosome.

A third myeloma analysed showed two totally different C Kappa gene arrangement. One of these fragments of DNA carried the complete rearranged V_K-J_K-C_K gene. The fact that the myeloma cells are highly aneuploid and malignant makes a reasonable interpretation of these experiments being hard to interpret since these

aberrant arrangements may reflect the neoplastic phenotypes of tumor cells and not any normal rearrangement of B immune lymphocyte.

Analysis of the intensity of the J Kappa C Kappa linkage segment on Southern blots (Joho and Weissman 1980) revealed that approximately 40 percent of C Kappa gene may be expressed in the germ line. Thus for the Kappa chain locus, the major contribution to allelic exclusion is via limited translocations.

Allelic markers do exist for the heavy chain chromosome as mentioned earlier . At present much effort is being made to determine the arrangements of V_H, J_H, D and C_H and C_H genes on the excluded chromosome to see if allelic exclusion is due to selective inactivation or activation, but no uniform picture has emerged (details Cold Spr Harbor Symp.Q.Biol.1976).

As discussed earlier under the section of 'a single variable region being shared by many constant regions' it was said that new techniques had helped us in our understanding of this mechanism. Here is a detailed account of the present understanding based on the organization of Heavy chain gene family.

Case - I: It was seen that majority of lymphocyte B cells express IgM and IgD simultaneously at the cell surface (Fu et al 1975) . Genetic analysis using cloning techniques performed showed that the C_μ and C_δ genes are linked and there exists no J genes in between and also in the DNA of IgM and IgD, a V_H-D-J_H joining at the J_H locus occurred. However, no rearrangement could be detected around the C_δ gene (Lin et al 1980) (Moore et al 1981) . This supports the concept of co-expression of IgM and IgD by a mechanism of what is claimed as 'differential RNA splicing' from a large RNA precursor molecule encompassing the entire V_H-D-J_H-C_μ-C_δ region (see figure 26A)(Alt et al 1980, Rogers et al 1980; Dildrop and Beyreuther, 1981).

Case II: Most B lymphocytes displaying these IgM and IgD molecules at the cell surface may differenciate when stimulated by antigen into plasma cells capable of secreting IgG1, IgG2, IgA and IgE molecules (Zanbar et al 1977a). That is, these cells posses the same antigen binding sites but different heavy chain families. This phenomena of genetic heavy chain switch (the same V_{L-H} region but different C_H regions) was puzzling until the structure of C_H region was actually understood.

Figure : 26-A : RNA Transcription and splicing are supposed to occur which account for succesive occurances of both membrane bound and secreted IgM and also for simulataneous production of IgD and IgM molecules.

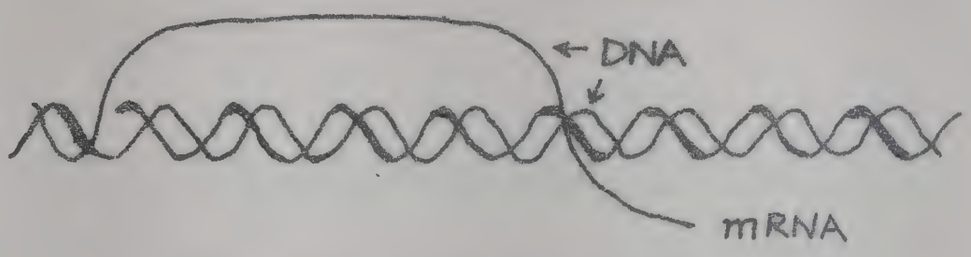
The diagram shows active gene organization with both Mu and Delta sequences. If transcription is terminated after 4th codon (as marked in the diagram)(A)then the RNA is spliced to yield a mRNA for a chain lacking the amino acid sequence responsible for anchoring to the cell . The IgM produced by this process is secreted.

B) : If transcript includes 5 and 6 genes then the stop codon of A is spliced and the 5th and 6th signals are included: they obviously are responsible for those amino acid sequence which anchor the Ig molecule to the membrane thus IgM produced by this process forms the membrane found molecule.

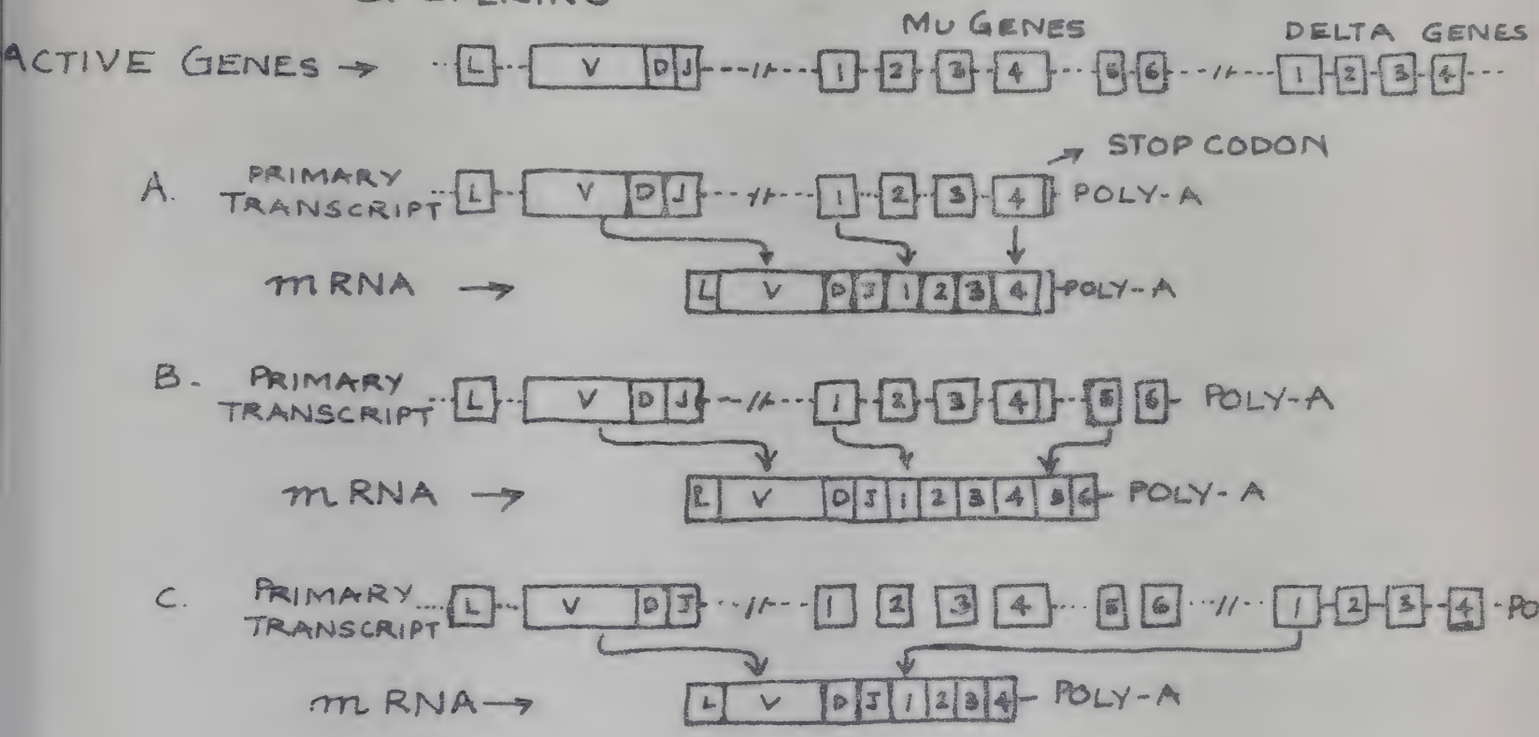
C) : Splicing also occurs to eliminate the mu genes and connect on the Delta sequence, thus resulting in the production of IgD

[adapted from Seidman J.C. and P.Leder (1978) Nature 276,790. and Siedman et al (1978) science 202; 11]

Figure : 26-B : Heavy chain 'class switch'.
The active heavy chain gene is shown in the figure .
It is made up of 5 gene signals but 8 gene segments in all (3+1 of Gamma) which form the C region gene and the other includes L/V, D and J . L-V-D-J segments are brought together by mechanisms explained earlier but the C region gene which as mentioned earlier is down stream separated by a noncoding sequence(UT). Each of the C genes are preceeded by a switch signal(s) having some complementary affinity to similar signal of sequence between the variable region sequence and Mu gene. This signal 'S' gene is thought to mediate a recombination between the L-V-D-J to any of the C genes. The switched DNA is transcribed and the RNA spliced to make mRNA encoding a complete Ig-X molecule [x = $\alpha, \delta, \mu, \varepsilon$ or γ_1 γ_{2a} γ_{2b}, and γ_3]



1. + RNA TRANSCRIPTION + & SPLICING



2. + HEAVY CHAIN CLASS SWITCH +

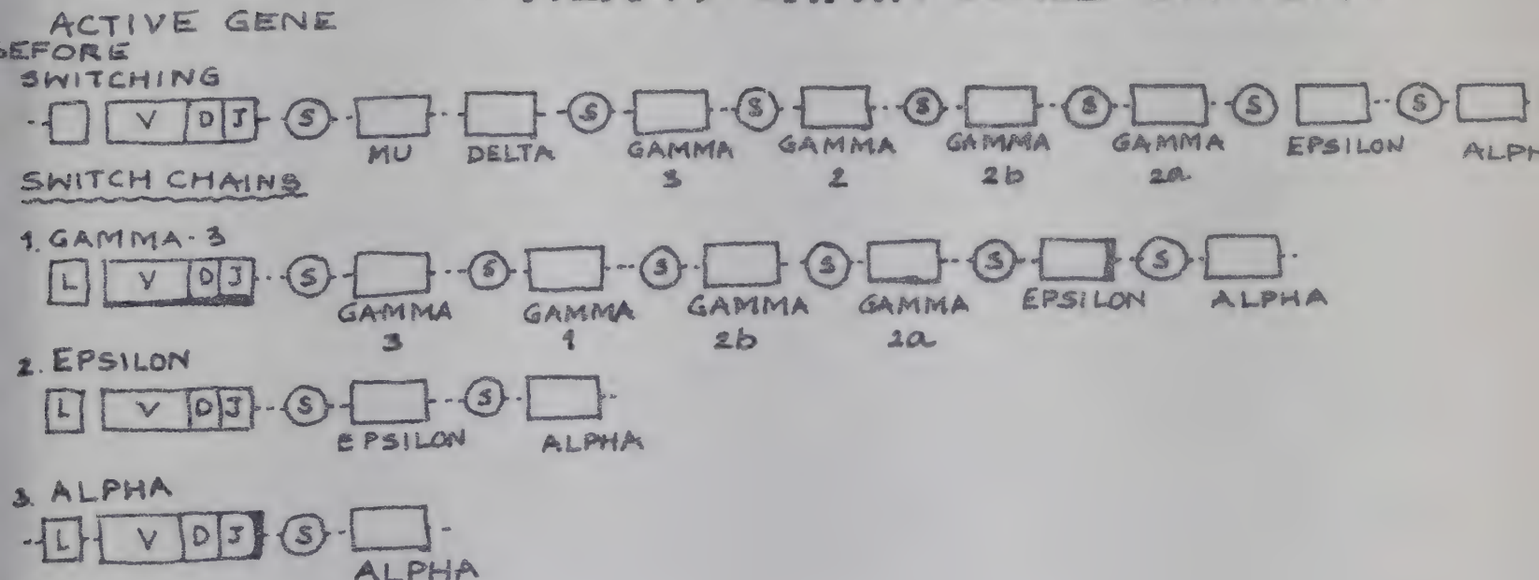


Figure - 26 (A & B)

The structure of C_H region was found out by two independent approaches. Most recent cloning of large overlapping sequences of DNA has allowed a direct physical linkage of C_H gene complex (Honjo et al 1981; Nishada 1981; Moore et al 1981; Liu et al 1980; Roeder et al 1981). The phenomena of V and C region translocation through a mechanism of 'DNA deletion' has led to the concept of several subsequent steps of DNA deletions briging into close proximity the V_H gene and C_H gene to be expressed. Since μ is the first isotype to be synthesized V_H-D-J_H joining activates transcription and expression of a μ chain gene. A second translocation of the complete heavy chain V region gene into close proximity with the C gamma 1 gene activates transcription of the gene (Kataoka et al 1980). Heavy chain gene located between J_H and C gamma 1 would be deleted (see figure 26B).

So the DNA deletion model of variable constant region rearrangement being recently proposed throws open the idea of gene rearrangement as a highly debatable issue awaiting experimental support.

It has been very recently reported (Richards et al 1983) that there exists unusual sequences in the murine IgM-D heavy chain region. This group claims

that they have sequenced the region of DNA between IgM-IgD inclusive of the sequence of the intrans occuring in the C-δ gene (intervening sequences). The analysis of these fragments have resulted in several interesting results. Of these the demonstration of the existance of a pseudogene in the large intron of C-δ resembling the switch(s) recombination sequences, associated with the above mentioned class switch mechanism in heavy chains (Early P e' al 1980), Davis M.M. etal (1980, Honjo T. et al.,(1982) and Honjo et al (1982 is welcoming / But the puzzling aspect is that no sequences reminiscent of switch sites was found when a 3 deletion end point of an IgD producing myeloma was analysed.

Apart from the above mentioned results, yet another interesting finding, which is a little deviation from the discussion in this chapter, but worth the mention, is that of some unusual sequence of the DNA.

Sequence analysis performed by J.E. Richards et al 1983 have shown multiple repetition of unusual sequences (bases) $(CA)_{24}$ $(TA)_1$ $(CA)_8$ referred to as $(CA)_{33}$ and it is supposed to be located between C_μ secreted as (μ_s) and membrane (μ_m) regions. $(CA)_{33}$

along with a few characteristic preceeding sequences could possibly form Z-DNA (Phol.F M and Jorin T.M.(1972).

The biological significance of the unusual sequences features found between C_δ and C_μ is not clear and yet speculated about. It is thought that at the chromatin level, the existance of Z-DNA, may play some role in the switch mechanism regulation

4.7. Immunoglobulin gene to immunoglobulin chain

Once the immunoglobulin genes are expressed in the functional form, they are to be transcribed i.e., the genetic 'message' of the immunoglobulin genes are now copied on to a ribonucleic acid sequence (RNA). Under the normal circumstances the genes of any biologically conventional protein, are transcribed into a RNA from the DNA, and later 'translated' to form the protein molecule (protein synthesis mechanism).

Not much was known, of the mechanisms involved relating to the intiation of transcription with respect to the immunoglobulin genes, until recently.

Since the immunoglobulin molecules are produced in large numbers; both qualitatively and quantitatively, it is seen that the Ig gene locus in a fully differenciated

B lymphocyte (Plasmacell) is transcriptionally very highly active. The transcription occurs at levels varying over a five fold rage compared to any other gene segments or locus.

Interestingly, a startling situation exists regarding the transcriptional capabilities of the immunoglobulin genes. It has been recently shown that there exists transcription enhancer elements in the intron between the J_H and C_μ segments of the heavy-chain immunoglobulin gene in mice [Gillies, S.D. et al(1983); Banerji, J., et al (1983) and Neuberger, M.S. (1983)] and were supported by experiments which showed the existence of enhancer-like elements for the mouse immunoglobulin light-chain J_K-C_K intron[Queen C and Baltimore, D.,(1983); Emorine, L., et al (1983) and Chung, S.Y., et al (1983)]. T.H.Rabbitts et al (1983); Mills, F.C., et al (1983) and Hayday, A.C. et al (1983) have shown that enhancers also occur in man.

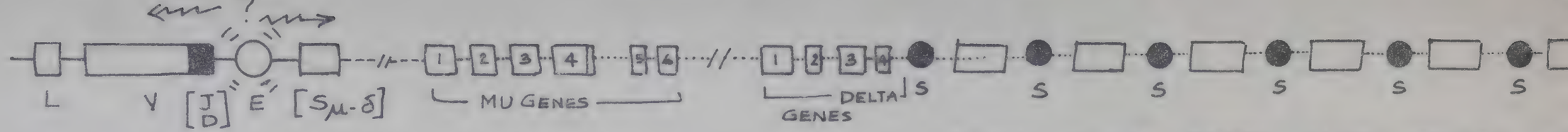
What are the functions of these enhancers? How do they help in transcirption of the immunoglobulin genes? [Incidently, enhancers were first identified in sequences near the replication origins of animal viruses like that of the polyoma virus [Khoury, G and Grauss,P(1983)]. Studies have shown that these viral enhancers in 'artificial genes'

dramatically stimulate transcription irrespective of the gene orientation], applying this with respect to the immunoglobulin gene enhancer, it is believed that the functionally active transcriptional enhancer, which is located upstream of the human C_μ gene, may explain why V genes are not only highly transcribed after they have been joined to J_H genes, even though germ-line. V genes possess active promoter regions [Bentley, D.L. et al 1982]. And it has been pointed out[Gillies, S.D., et al (1983) and Banerji, J. et al (1983] that, since the enhancer element is located between J_H and S_μ they allow the sequence to be maintained near each C_H gene, which are successively expressed during switch. Thus it follows that the enhancers are tissue specific and could possibly result in a selective basis of expression, of the rearranged V region in B lymphocytes. The mechanism of enhancers is not yet understood. However, several hypothesis have been postulated explaining the ability of the enhancers to control regional transcription irrespective of their orientation and location. One such proposal, suggests that the enhancer sequences act as entry points for RNA polymerse (compenent of protein synthesis). and/or other transcription factors. The idea is made more picturesque with the suggestion that enhancers are involved in opening up the local

chromatin structure thus making, not only them, but also the immunoglobulin genes more accessible to the cells transcription apparatus. [see figure 27]

But recent findings, have resulted in controversy regarding the actual role of enhancers, Picard, D and Schaffror, W (1983) have shown that enhancer segments do not occur associated with the lambda light chain immunoglobulin genes. The other finding which has created ambiguity, is with respect to the expression of the oncogenes [incidently oncogenes are genes which are said to cause cancer. These genes occur in every normal cell, but when they are expressed in a abnormal fashion they result in cancerous growth], which are involved in the tumor called Burkitt Lymphoma. A specific oncogene known as myc gene. (myelocytomatosis virus gene is said to be responsible for the lymphoma cancer. The malignant cell undergoes specific chromosomal rearrangement involving the region proximal to the myc genes and that of the immunoglobulin genes. The rearrangement finally results in a translocation of the myc genes to a site which is normally the residence of the V region genes of the Ig. The nature of the effect exerted by the immunoglobulin genes is controvertial: whereas some reports have suggested myc transcription is elevated in the lymphoma cells [Erikson, J., et al (1983)]

HEAVY CHAIN TRANSCRIPTION ENHANCER

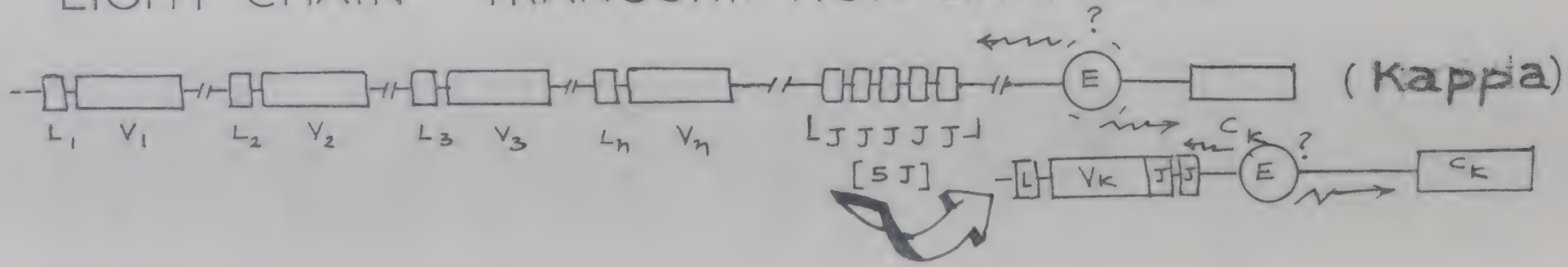


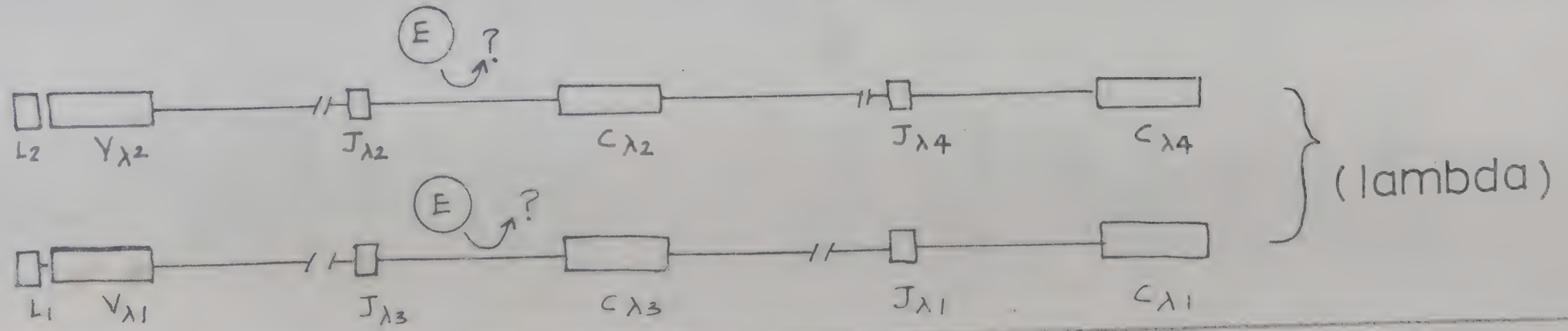
L: LEADER
V: VARIABLE
J: JOINING
D: DIVERSITY

E: ENHANCER SEGMENT (TISSUE SPECIFIC)

S: SWITCH GENE (CLASS SPECIFIC)

LIGHT CHAIN TRANSCRIPTION ENHANCER





others have suggested the level of transcription is uneffected by translation [Hamlyn P.H. et al (1983)]. The story takes a twist when it was shown that in case of Burkits lymphoma, the maglignant cells translocate the 'essential' immunoglobulin enhancer from the abnormal chromosome [the myc-Ig genes linkup] to elsewhere and it cannot influence the transcription of the translocated myc gene, but the fact remains that in another tumor known as Manca B lymphoma cells, the myc transcription is very much dependent upon the immunoglobulin enhancer.

To sum up, it may only mean that a third mechanism(s) is in any case required to explain the increase in transcription that accompanies the production of secreted immunoglobulins.

Irrespective of the above result, the 'next step' is the process of transcription of the immunoglobulin genes.

The V and C region nucleotides in light chain mRNA corresponds exactly to the amino acid sequences of the light chain. the mRNA also includes nucleotides for the leader sequence found on nascent chains and in addition a further 200 nucleotides on either end. Since the mRNA are not supposed to have introns which occur at the DNA level. There must therefore be a mechanism where by the introns are omitted from cytoplasmic mRNA.

The mRNA in the cytoplams is derived from a much larger RNA sequence present only in the cell nucleus and are known as heterogenous nuclear RNA (hnRNA) (Rabbitts et al 1978). It is believed that the hnRNA undergo post transcriptional modification, the introns removed and a poly A added to the tail region [see figure 25]. The mRNA resulting from the above hnRNA is now translated to produce the appropriate immunoglobulin molecule.

STRUCTURE OF κ mRNA

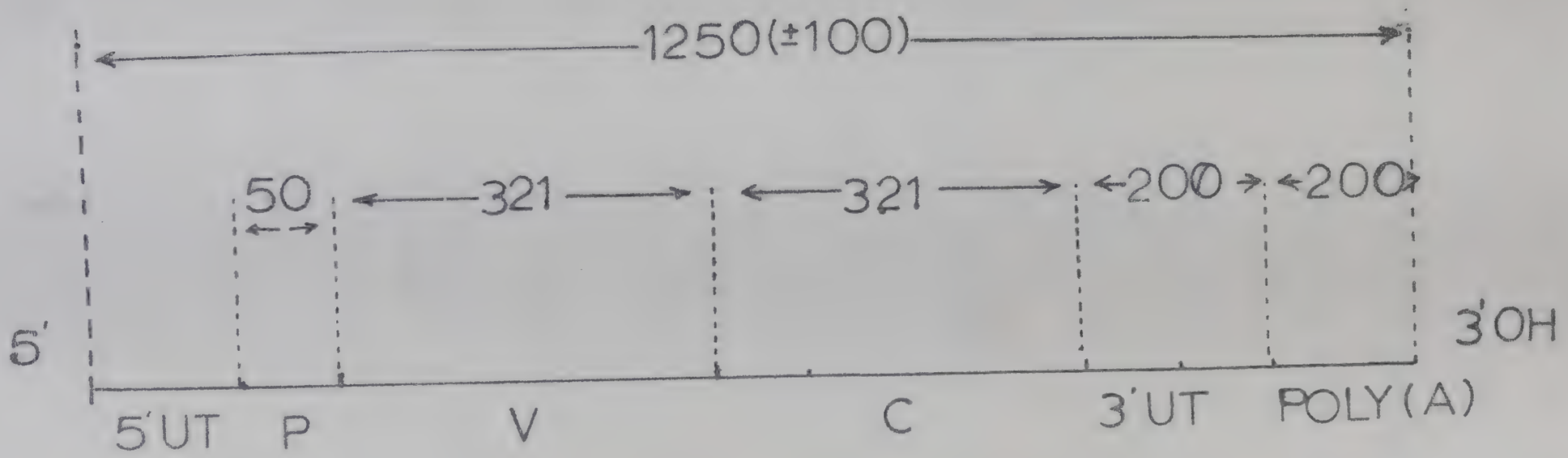


Figure - 28

4.8. Immunoglobulin genes : as in Lymphocytes

So far, what ever that has been described regarding gene organization, generally holds true for the B lymphocytes. This is so because only the B cells secrete immunoglobulin molecules. The T cells on the other hand, which play the central role in cellular response, also recognize antigenic molecules. Contrary to B cells where the antigen receptor is well known (membrane bound immunoglobulin) the T cell receptor has not yet been identified and characterized. [Warner, 1974 and Rajewsky and Eichmann, 1977].

There exists a lot of ambiguity regarding the gene organization and expression of these surface receptor molecules. [Marchalonis et al 1980; Kemp et al 1980a, 1980b, Walker and Harris 1980; Kuronenberg et al 1980; Kurosawa 1981]. For details regarding genetic origins of B and T lymphocyte immunoglobulins refer to Tonegawa S et al(1983b).

In conclusion, an interesting aspect could be raised: what are the total number of immunoglobulin genes that exists? the question with which we began actually our discussions. The answere is that it is 'evidently uncertain' and still eludes the biochemists. Again two theories have been proposed for the origin of the DNA coding for immunoglobulins.

(a) Germ-line hypothesis - arguing that all the immunoglobulin genes are inherited and that antibody diversity or production is a result of mutation. [Hood et al., 1975].

(b) The somatic mutation model proposes that diversity is generated by somatic mutation from a small number of inherited V region genes during the life term of the organism. [Bernner and M.Istein, 1966; S.Tonegawa et al., (1983)) Weigert et al., 1970].

This topic has generated two important and interesting avenues.

a) the creation of a new topic for discussion and the problem of evolution and diversity of immunoglobulin molecules - related to immunoglobulin genes themselves.

b) support for either hypothesis rests on the question itself; i.e. the estimation of the number of inheritable V region gene. This appears to be a tough task for the present.

A substantial work has been done in order to improve one understanding in this field, but there is no doubt about the fact that a lot more exists which have to be discovered and understood!

Conclusion

It is really a fascinating experience as one tries to picture his or her own immune system. A wonder that surpasses all those wonders seen through the naked eye. It is also equally puzzling, how magnificantly every organisms immune system works in order to maintain the exclusive body equilibrium. As it is well known that recognition plays a central role in life any entity which can perform recognition at all levels are not only complex but make themselves much superior to those which cannot perform this function. Recognition whether it is the capacity of an enzyme to recognize its substrate or a tRNA to that of the cognate codon in mRNA or an whale being capable of recognizing its mate, but the immune system of the vertebrates has the capacity to recognize entities unmatched to any of those which can perform this function!

Thus using this trump card ,the immune system's agents like the lymphocytes patrol the vertebrate body reading every entity that is present around it and checking out if the entities belong to the co-ordinated network of organism (thus self) which it 'tolerates' or any foreign agent ready to disturb this co-ordination and create problem. Those with unfamiliar 'looks' are totally

destructed and removed.

As we have seen, the body's defense network is certainly a complex organization of cells, cell types and organs , this review covered only a small area in the field of immunology. Apart from immunoglobulins and their role in immune mechanism, there are other frontiers in this branch of science which are of much more complexity and uncertainity with respect to our understanding of them. The mostchallenging of these are the major Histocompatibility system which plays the role of recognizing 'self' from 'nonself', thus playing the key role of the immune system. They co-ordinate the functions performed by the immune-agents. The important reaction mechanism of MHC system is 'what they see of others' (be it cells, tissues molecules) 'not how they see' entities!

To sum up the story,it is believed that the vertebrate lymphatic system's macrophages process every antigen within the body system. The processed antigen are presented to a specific T cell, which reads the antigens surface structure. There is an interesting aspect here. It is believed that all non-complementary structures displayed by entities (be it displayed by an external agent or even by a cell belonging to the organism) the T cells read these

configurations as nonself and mount reaction against them. Such is the arrangement of the immune system and that of the other cells of the organism, consider for example a viral attack. The cells devouring (or invaded by) virus, display on their surface not just the proteins alone made by the virus, but the viral proteins are so arranged on the cell surface, that they alter the self antigen which the infected cells possess. The T cells read the altered self antigen as 'nonself' and kill the cell belonging to the organism, containing within it the virus. It is certainly amazing to see how an innocent cell betrays itself and gets killed in order to maintain integrety of the organism! How does this work?

> And the end of all our exploring
> will be to arrive where we started
> and know the place for the first time.
>
> -T.S.Elliot
> Little Gidding

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